EXINE STRUCTURES IN SOME FOSSIL AND RECENT SPORES AND POLLEN AS REVEALED BY LIGHT AND ELECTRON MICROSCOPY

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EXINE STRUCTURE IN SOME FOSSIL AND RECENT SPORES AND POLLEN AS REVEALED BY LIGHT AND ELECTRON MICROSCOPY

By JOHN MICHAEL PETTITT

CONTENTS

SYNOPSIS

This paper gives descriptions of the wall structure of the spores and pollen grains from a variety of living and fossil plants.

The spores of Palaeozoic heterosporous plants are compared with those of extant homosporous and heterosporous pteridophytes, and Carboniferous pteridosperm and cordaite pollen is compared with that of living cycads and conifers.

It has been found that the structure of the megaspore membrane in some Palaeozoic gymnospermous ovules more closely resembles the spore exine of some free-sporing heterosporous plants than the megaspore membrane of living gymnosperms. Similarly, the exine structure of pteridosperm and cordaite pollen is closer to that of some pteridophyte spores than to the pollen of living gymnosperms. Special attention has been paid to the origin and distribution of the various wall layers, particularly the mesospore and the perispore.

I. INTRODUCTION

The discovery by Ehrlich & Hall (1959) that fine structural detail can be preserved in the exines of Eocene angiosperm pollen has prompted Larson & Lewis (1961) to suggest that the evolutionary development of the pollen wall could be subjected to direct study.

More recently it has been shown that *Classopollis*, a Mesozoic conifer pollen possesses a distinctive wall structure, the complexity of which is unmatched even among living angiosperms (Pettitt & Chaloner 1964).

Afzelius (1956) has investigated the structure of the exine in the spores of living pteridophytes and the pollen of living gymnosperms and angiosperms, and has

suggested that certain submicroscopic structures in the exine may be more primitive than others. She suggests that a pollen grain with a thin lamellated layer in the exine may be considered phylogenetically more advanced than one with a thick lamellated layer. Larson & Lewis (1961) discuss this hypothesis and advance the suggestion that fine structural variations in the exine could be related to functional differences. Such a hypothesis would appear to be open to experimental verification, and if it is eventually substantiated any phylogenetic interpretations based on exine structure alone would have to be qualified accordingly.

It has been found that exine fine structure can be preserved in the fossil spores of free-sporing Palaeozoic pteridophytes and in the fossil pollen of Carboniferous seed plants, and that the stratification revealed in ultra-thin sections can be directly compared with the exine structure in the spores of extant pteridophytes and the pollen of living gymnosperms. The following is an attempt to synthesize the information so far obtained.

II. METHODS OF INVESTIGATION

The fossil material was released from the enclosing sediment with cold commercial (40%) hydrofluoric acid. Where necessary dilute nitric acid was used to clean and soften the spores and pollen prior to dehydration and embedding. Details of the fixation, embedding and histological procedures used in this investigation are given in Pettitt (1966).

Ultra-thin sections were cut either on a Huxley-pattern or a Reichert ultramicrotome using glass knives and mounted on copper grids with or without carbon reinforcement for observation with a Siemens' Elmiskop 1a or an A.E.I. EM6B electron microscope operating at 60 kV. Some use has been made of section stains and where they have been employed the fact is recorded in the figure legends.

Thicker sections (about 0·2 μ) of methacrylate and epoxy resin embedded specimens examined under phase and anoptral contrast illumination have been used fairly extensively in the investigation of the thicker exines.

The terminology adopted for the exine subdivisions revealed in the spore and pollen walls follows that proposed by Faegri (1956). Although this system of nomenclature was applied to explain the subdivisions detectable in the exine of angiosperm pollen following alcoholic basic fuchsin staining, Larson (1964) by using electron stains has shown that it is probably also applicable to the spores and pollen of non-angiospermous plants.

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III. DESCRIPTIONS

SPORES OF LIVING AND FOSSIL HETEROSPOROUS PTERIDOPHYTES

Spores of *Archaeopteris* cf. *jacksoni* Dawson

The occurrence of heterospory in this Devonian species has already been described (Pettitt 1965) and a full description of the morphology of the spores is given in that publication.

Megaspores. The subdivision of the megaspore exine into an outer granular layer and an inner apparently homogeneous layer has been detected with the optical microscope (Pettitt 1965). At that time it was recorded that some, but not all, of the megaspores possessed a conspicuous inner body or mesosporium. It was not possible, however, to see how the inner body was formed nor to determine precisely how it was related structurally to the remainder of the spore wall.

Ultra-thin sections of the megaspores from a sporangium of *A.* cf. *jacksoni* examined in the electron microscope support the interpretation based on the observations with the optical microscope. The thin, inner layer (endexine) which appears homogeneous in sections under the light microscope is seen to have a lamellated structure, whilst the outer optically " granular " layer (ektexine) is composed of three-dimensional, anastomosing sporopollenin units giving the layer a spongy appearance (Pl. 2, figs. 1, 2 ; Pl. 3, fig. 3). The outer spongy layer accounts for the greater part of the thickness of the wall.

The subdivision of the inner lamellated layer or endexine into a series of concentric lamellae is evident in all the micrographs. In some sections there is partial or complete separation of this layer into an inner and an outer zone (Pl. 3, fig. 3). The separation would appear to be along one of the periods between the lamellae and the fact that it does not occur in all the megaspores, together with the observation that the actual site of the separation within the layer varies in the different spores would suggest that it is not a regular feature of ontogeny.

From the evidence it seems reasonably certain that the separation of the endexine in these megaspores is responsible for the presence of the inner body or so-called mesosporium that can be observed in entire spores examined by transmitted light (Pettitt 1965).

In the earlier publication I provisionally assigned the megaspores of *A.* cf. *jacksoni* to the megaspore genus *Biharisporites* Potonié (1956) using the presence of a mesospore in some of the specimens as one of the features indicating a close correspondence with the spores of that genus. However, the discovery that the mesospore of these fossils is probably formed by the artificial separation of the inner layer of the exine into two immediately calls to question the validity of the feature as a taxonomic character.

The structure and formation of mesospore-like bodies in the spores of other fossil plants and in the spores of living pteridophytes is discussed later.

Electron micrographs of sections through *Archaeopteris* megaspores that are situated at the outside of the sporangial mass show an unusual feature. On the surface of these spores dense aggregations of droplets occur in close association with the underlying spongy layer of the exine. The droplets vary in size, and consist of an outer stratum of homogeneous sporopollenin which becomes granular towards the centre of the body. The droplets have a hollow central lumen which is traversed by irregular wefts or threads of material with a coarsely granular fine structure (Pl. 2, fig. 1). There is no evidence of structural connexion between any of these bodies and the outer part of the exine, and they cannot be regarded as part of the wall ornamentation in the strict sense. Recent observations on the formation of sporopollenin droplets in the degenerating tapetal cells of gymnosperm pollen sacs (Pettitt 1966) leaves little doubt that the droplets in the *Archaeopteris* sporangia have a similar origin. Rowley (1963) has called somewhat similar droplets Ubisch bodies in the pollen sacs of angiosperms, but as Ubisch bodies seem to have a functional role in pollen wall formation in these plants it would be unwise to apply the name to the droplets in the *Archaeopteris* megasporangia. They are perhaps more comparable to the objects which Ueno (1959, 1960) has described as the perine in the pollen of some gymnosperms. This point is more fully discussed in a later section.

Finally, it is evident that such deposits on the outer surface of the spore exine could easily be mistaken for sculptural elements in an uncritical examination.

Microspores. The microspore exine of *Archaeopteris* cf. *jacksoni* is divided into two distinct layers which, in their ultrastructure, closely resemble the layers of the megaspore exine. The inner layer (endexine), which lines the lumen of the spore, is lamellated, whilst the outer layer (ektexine) is composed of ramifying units of sporopollenin giving the layer a spongy appearance (Pl. 3, fig. 1). In addition to the fine structure and stratification of the exine, thin sections through the spore wall show some other features that warrant description.

The electron micrograph shown on Pl. 3, fig. 2 is of a section which passes through one of the triradiate commissures. The section clearly shows that the lamellated endexine passes upwards through the commissure and forms a lining to the aperture. On the surface of the spore the endexine overlaps on to the outer layer of the exine and gradually thins out. It seems probable that the structures just described are lips, although lips surrounding the triradiate laesurae of these microspores were not detected with transmitted light (Pettitt 1965).

The exine ornamentation of the microspores of *A.* cf. *jacksoni* has been described as being composed of minute conical elements about 1–1·5 μ in height, and 1 μ or less wide at the base (Pettitt 1965), and on the basis of this they were provisionally assigned to the genus *Cyclogranisporites* Potonié & Kremp 1954. The electron micrographs show how the conical elements are constructed from the sporopollenin units forming the outer exine layer (Pl. 3, fig. 1).

The spores of *Selaginella* and *Isoetes*

Megaspores of Selaginella. The electron micrographs of the acetolyzed megaspore of *Selaginella selaginoides* published by Afzelius, Erdtman & Sjöstrand (1954) show that the wall is composed of a " three-dimensional network of rounded bars ". An almost identical fine structure has recently been observed in *Selaginella myosurus* and *Selaginella kraussiana* by Stainier (1965), and from the observations made during the present investigation it would seem that the outer layer of the megaspore exine in a number of *Selaginella* species is of this composition. The following description is, however, based mainly on the megaspores of *Selaginella pulcherrima* Liebm.

Sections of the megaspore wall examined by phase contrast illumination show that there are structural variations within the outer exine layer. These are evident as concentric zones in the ektexine that appear to result from variations in the amount of interstitial space between the elements composing this layer of the wall. Electron micrographs show that at the outer edge of this outer layer the sporopollenin units become discontinuous and the wall texture is more open or porous in appearance. Towards the inner limit of the layer the sporopollenin elements gradually become reduced in thickness and less widely spaced and the boundary is seen as a zone of more compact structure (Pl. 4, figs. 1, 2). Martens (1960) and Stainier (1965) have detected obvious zoning in the megaspore exine of *S. myosurus* and they find that the sporopollenin units in certain parts of the exospore have a definite orientation which would seem to be unique to this species.

The acetolyzed megaspore wall of *Selaginella* consists mainly of a very thick, spongy outer layer or ektexine. On the inside of this, however, is a much thinner layer, the endexine, which accounts for not more than one-twentieth of the total thickness of the exine. Although the endexine has not been adequately photographed in the electron microscope it can be clearly seen in thin sections that were examined and photographed by anoptral contrast illumination (Pl. 5, fig. 1). In these sections the endexine appears to be faintly lamellated, and the junction between it and the ektexine is very distinct.

Fitting (1900) published a very detailed account of megaspore development and structure in *Isoetes* and *Selaginella*. As his observations are of significance to the present study the more relevant of them should perhaps be briefly reviewed.

Fitting recognizes three or sometimes four layers in the megaspore wall of *Selaginella*. The outer layer in some species is a thick silicified " perispore ", but this is not always present. Underlying the perispore is the exospore which is often differentiated into two layers. Within the exospore is the mesospore, a very thin, yellowish membrane that is easily separated from the overlying layer. The innermost layer recognized by Fitting is a cellulosic endospore.

Fitting followed the development of the sporoderm in *Selaginella* megaspores and sets out a very precise series of events. He found that the exospore is the first formed layer and that this increases in thickness to between 0·5–1·2 μ (depending on the species) before the mesospore is formed. According to Fitting the mesospore is formed as a new layer and does not arise as a result of splitting in the exospore. The fact that from its inception there is sometimes a gap between the

mesospore and the exospore is advanced as evidence for formation *de novo*. Following its formation the mesospore rapidly increases in thickness.

In this connexion it is worth noting that Fitting observed two rather relevant differences in the megaspores of *Selaginella galeotii* on the one hand and *Selaginella spinulosa* and *Selaginella helvetica* on the other. In the first species a distinct margin between the exospore and the mesospore is not evident, whilst in *S. spinulosa* and *S. helvetica* there is a division in the middle of the exospore extending from the proximal to the distal surface which divides the layer into two membranes of equal thickness.

Fitting noticed that as the megaspores increase in size the exospore and the mesospore become separated from each other and from the spore protoplast. The layers remain firmly attached at the spore apex and the separation can be detected first in the distal region of the spores and later in the equatorial region. There is apparently some variation in the stage at which this separation occurs and in the extent to which it finally develops. An extreme example would seem to be the megaspores of *S. spinulosa* where the two layers remain firmly attached at all times. In the megaspores of some species bars or trabeculae, which have the same composition as the spore wall, were seen to link the exospore and the mesospore together. These structures are rather short-lived, and with the increase in size of the spores they become thinner and finally rupture. Fitting regards the presence of the trabeculae in *Selaginella* as indicative of close connexion between the exospore and the mesospore in young megaspores, and he believes that they originate as a result of polymerization or coalescence of the two membranes at their points of contact. The trabeculae are formed at the sites of contact as the membranes draw apart during spore growth and the space between them increases. The space between the two wall layers and between the mesospore and the spore protoplast is filled with an interstitial substance (more evident in older spores) which Fitting found to give a pectin reaction and also to stain with Congo red and Analine blue.

The interstitial substance gradually disappears as the megaspores mature and the mesospore expands until it makes contact with the exospore. Both layers then grow in circumference with the result that in the mature megaspores the thick mesospore present in the early stages of development is present as a thin membrane closely associated with the exospore.

Somewhat later the cellulosic endospore is formed and the protoplast increases in mass and fills the spore lumen. About this time the perispore is formed from the lamellae of the special mother cell wall.

Of the two papers published by Lyon (1901, 1905) describing exine formation in *Selaginella* the latter is the most pertinent to the present account. In this, Lyon questions some of the conclusions reached by Fitting (1900) and revalues the earlier work of her own. Lyon detected only two megaspore wall layers in the material she investigated; the exospore, which is formed first, and the endospore, which is lamellated. Some confusion arises here because the layer that Lyon (1905) calls the endospore is the layer which Fitting (1900) calls the mesospore. Lyon does not find a mesospore *sensu* Fitting in her material.

Lyon's investigations lead her to conclude that the exospore and the endospore (mesospore of Fitting) are sometimes formed simultaneously, and that the space which occurs between these two layers is an artifact caused by shrinkage. Lyon's evidence for this suggestion is based on the observation that in some megaspores the membranes do not separate. Lyon also finds that in young megaspores there is a tendency for the exospore to split into an outer and an inner layer and that the inner part of the exospore, to which the endospore is attached, could be interpreted as a separate wall layer.

Campbell (1902) suggests that the cavity between the wall layers of the megaspores originates as a result of the exospore enlarging more rapidly than the mesospore. According to this author the mesospore closely resembles the exospore in appearance and could arise by the separation of an inner layer from the exospore.

More recently, Stainier (1965) has recognized four wall layers in the megaspore of *S. myosurus*, which correspond to the four detected by Fitting (1900), and three in *S. kraussiana*. This author finds that the mesospore is separated from the exospore in both these species and concludes that the separation in *S. myosurus* is a natural phenomenon which occurs during spore growth but in *S. kraussiana* it is probably a fixation artifact.

The descriptions given by Fitting (1900), Lyon (1905), Campbell (1902) and Stainier (1965) show that whilst there is some measure of agreement in the naming of the various exine subdivisions that these authors detected in the megaspores of *Selaginella* it is far from being absolute. It is difficult, for example, to accept that the layer which Fitting describes and illustrates as the mesospore in young *Selaginella* megaspores is identical with the layer to which Campbell gives the same name. It is apparent that the term mesospore is being applied to very different structures.

The importance of an inner body or mesospore as a microscopical character in the recognition of a number of fossil microspore and megaspore genera has already been mentioned. There is some difference of opinion as to whether this structure is an artifact (see Kremp 1965) and the conflicting evidence from living material hardly helps to resolve these differences. It is of some interest, therefore, to try to establish the true nature of the mesospore.

Sections of *S. pulcherrima* megaspores containing mesospores of the type illustrated by Fitting (1900, pl. 6, fig. 23) in his immature megaspores were subjected to a variety of histochemical tests. It should be pointed out that possibly only one or two of the four megaspores in any one sporangium contained mesospores of this type. Judging solely from their external appearance all the spores at first seemed to be perfectly normal. However, sections showed that normal protoplasmic contents, whilst present in some of the spores, are completely lacking in the spores that contain a mesospore. This observation completely contradicts those of Fitting (1900) and Lyon (1905).

Structurally the central part of the mesospore appears fibrous under the light microscope at high magnifications and the fibrosity gives way to a more granular structure towards the periphery of the body. In the centre is a hollow cavity (Pl. 5, fig. 4). Trabeculae linking the mesospore with the ektexine (exospore of

Fitting) are sometimes present. The endexine could not be detected in megaspores that contained a mesospore.

Sections stained with Sudan black B show that the entire mesospore and the ektexine are strongly sudanophilic (Pl. 1, fig. 5). Following treatment by the periodic acid/Schiff (PA/S) procedure the central, fibrous zone of the mesospore gives a vivid red colour, but there is no reaction from the outer granular zone, nor from the ektexine (Pl. 1, fig. 3). This staining pattern is repeated in sections stained with ruthenium red. It is, however, reversed in sections stained with Nile blue sulphate. The inner fibrous zone stains faintly, if at all, whilst the outer granular zone of the mesospore and the ektexine stain a strong blue colour (Pl. 1, fig. 4). From these tests it can be reasonably concluded that the inner fibrous zone of the mesospore gives positive indications of the presence of lipids and carbohydrates.

Further characterization of the nature of the body was obtained after treating sections with mercuric bromophenol blue, developed as a protein indicator by Mazia, Brewer & Alfert (see Pearse 1961). It was found that the ektexine and the outer granular layer of the mesospore remains unaffected by this test, but that the inner fibrous zone is coloured faintly blue-green (Pl. 5, fig. 3).[1]

As a measure of control, sections of megaspores that did not contain a mesospore were subjected to the same histochemical procedures. Following acetylated Sudan black B staining the spore protoplast colours strongly and three sudanophilic wall layers are recognizable; the ektexine, the endexine which is frequently detached from the ektexine except at the spore apex and a thin membrane closely associated with the spore protoplast. The identity of this last layer is only clearly recognizable where the protoplast has become separated from the outer wall layer (Pl. 1, fig. 6). At the apex of the megaspore, where cellular differentiation has occurred, the membrane is seen to have anticlinal walls which closely match the position of the cell walls of the underlying gametophyte tissue. So far, this layer has been detected only in the megaspores of *S. pulcherrima* and in this respect these spores would seem to be unique.

Between the membrane just described and the endexine is an optically structureless layer that does not colour with the Sudan stain but which stains intensely with ruthenium red. This layer, which is not always detectable can, by analogy with pollen wall terminology, be called the intine and is presumably that which Fitting (1900) and Stainier (1965) call the endospore.

The ektexine is coloured bright blue after Nile blue sulphate staining, whilst a subtending layer, which from its position would appear to be the intine, stains a strong purple colour. The blue coloration of the ektexine following the Nile blue sulphate method indicates that this layer contains a high proportion of acidic lipids, whilst the purple colour of the inner layer following the same test would suggest that this contains predominantly neutral lipids. The discovery that the inner layer is composed of pectic substances that are stained with ruthenium red and neutral lipids is somewhat surprising, and perhaps the analogy with the pollen

[1] It has proved impossible to obtain a colour-faithful photomicrograph of this test and, consequently, it has not been included in Pl. 1.

intine extends only to the location of the layer and not to its composition. Stainier (1965) has shown that the endospore of *S. myosurus* is cellulosic.

Following section treatment by the PA/S test the intine and the thin sudanophilic membrane that surrounds the protoplast give a strong red colour characteristic of a positive Schiff reaction. The protoplasmic contents of the megaspore stain faintly (Pl. 1, figs. 1, 2).

Preparations treated with mercuric bromophenol blue reveal the presence of proteins in the megaspore protoplast which is coloured blue-green after this procedure (Pl. 5, fig. 2, see footnote on p. 230). No reaction is seen from the megaspore wall layers.

The evidence from these histochemical methods strongly suggests that the mesospore, *sensu* Fitting, in these spores is not composed of sporopollenin. Although the presence of lipid in the ektexine, the endexine and the mesospore is clearly demonstrated by Sudan black B staining, the mesospore, or more correctly the central zone of it, also gives positive reactions for carbohydrates, pectin and proteins. Carbohydrates, pectin and proteins were not detected in the ektexine or endexine, but carbohydrates and proteins do occur in the normal megaspore protoplast. The only conclusion that can be drawn from these results is that the mesospore is chemically more closely allied to the spore protoplast than to the exine.

The occurrence of the mesospores in megaspores without a recognizable protoplast together with the histochemical evidence suggests that the inner fibrous zone of the mesospore represents an abortive protoplast that has undergone serious deformation at some stage during its development. The outer granular zone of the body behaves chemically like the exine material and the presence of this layer may be correlated with the apparent absence of an endexine in these spores. Presumably the resistance of the mesospore to acetolysis could be explained by the presence of the lipid and protein.

An entirely different interpretation can, however, be placed on the structure which Fitting (1900) and Stainier (1965) designate the mesospore in mature spores of *Selaginella*. In mature megaspores of *S. pulcherrima* and *Selaginella padangensis* Hieron. with normally developed protoplasts the endexine is frequently found detached from the ektexine. This is clearly seen in sections of acetolyzed megaspores where the endexine is present as a distinct inner layer, separated from the ektexine by a narrow cavity, and enclosing a central lumen. These preparations also show that the ektexine is frequently split, particularly in the apical region, a phenomenon to which Lyon (1905) draws attention. Sections through the apical region of the megaspores show that the endexine is attached to the ektexine in the region of the triradiate sutures in precisely the way that Fitting (1900) describes for his " exospore " and " mesospore ". Not infrequently it can be seen attached over a much greater part of its circumference. The frequent apical attachment of the endexine to the ektexine is explained by serial sections cut tangentially through the proximal region of the megaspores. These show that the endexine passes up into the triradiate commissures of the spore in exactly the same way as in the microspores of *Archaeopteris* (Pl. 5, fig. 5).

It would appear that the mesospore recognizable in the normal, mature and presumably viable megaspores of *Selaginella* is in no way identical to the structure given that name by Fitting (1900) in the spores which he believed to be immature but which are probably abortive. In normal, mature *Selaginella* megaspores the mesospore is formed by the endexine separating from the ektexine around most of its circumference as suggested by Stainier (1965) for *S. kraussiana*, but remaining attached to the outer layer at the triradiate laesurae. Fossil megaspores containing precisely this type of structure are described in the previous section. In contrast, the central body or so-called " mesospore " of abortive *Selaginella* megaspores is probably a protoplasmic remnant which has undergone considerable structural deformation. Whether or not this structure which will survive acetolysis and oxidative maceration would also survive fossilization is questionable.

Microspores of Selaginella. Ultra-thin sections of microspores of *S. pulcherrima* show that the entire thickness of the exine is composed of sporopollenin that appears homogeneous or faintly fibrous in the electron microscope (Pl. 5, fig. 6). There is no evidence of an inner lamellated endexine. The fine structure of the microspore wall of this species is, therefore, very different from that of the megaspore.

Surrounding the entire microspore and interrupted only at the triradiate commissures is a thin, electron-opaque layer which has a somewhat granular ultrastructure. In the megaspores of the same species a well-demarcated, phase-white, granular layer can be seen on the outside of the exine when sections are examined by anoptral contrast illumination (Pl. 5, fig. 1). Fitting (1900) describes a granular silicified layer on the outer surface of the *Selaginella* megaspores to which he gives the name " perispore " and it is reasonable to assume that the granular electron-dense and phase-white layers in *S. pulcherrima* could bear the same interpretation. The optical appearance of the layer suggests that it is an inorganic substance (probably silica) that has been deposited on the outer surface of the exine. The question of whether this should rightly be called a " perispore " in the sense that the term has now been defined is discussed later.

Following histochemical tests with Sudan black B, Nile blue sulphate, PA/S and mercuric bromophenol blue the microspores of *Selaginella* behave in much the same way as those megaspores lacking a mesospore. The exine is seen to be sudanophilic and colours blue after treatment with Nile blue sulphate, but gives no reaction after the PA/S test. The siliceous " perispore ", however, of both the microspore and megaspore stains vividly with celestin blue (Pl. 1, fig. 2). The spore protoplast is stained faintly red after PA/S treatment, and a strong blue-green colour after section treatment with mercuric bromophenol blue. The microspore exine is not coloured by the protein test.

Megaspores of Isoetes. In his account of the structure of the megaspore of *Isoetes* Fitting (1900) divides the wall into four layers which are, from the outside inwards, a strongly silicified perispore, an exospore (which is often split into three sub-layers), a thin mesospore and a cellulosic endospore. The extent to which the perispore is developed varies somewhat in different species and it forms the ornament

in those megaspores which are ornamented. The ornamentation is repeated in the underlying exospore. There is, therefore, a close association between the perispore and the exospore in *Isoetes* and some interbedding is not uncommon. Fitting's study shows that the perispore is laid down only when the megaspores are mature.

In most species of *Isoetes* Fitting finds that the exospore is clearly divided into three subdivisions or lamellae. This feature is particularly clear in those species which have a thick exospore, and apparently transitions exist within the genus from spores in which exospore stratification is clearly recognizable to those in which none can be seen.

Fitting describes the exospore of *Isoetes* as being composed of small, dark brown " crystalline " rods which are arranged parallel to the surface of the spore. The rods are more closely packed in the middle zone of the layer than in the outer or the inner zones.

The mesospore is described as a thin layer that in both young and mature spores is always somewhat separated from the exospore and which has the same chemical composition as the latter.

The cellulosic endospore is intimately connected with the mesospore which, according to Fitting, exhibits some stratification following treatment with cuprammonium. Between the endospore and the spore protoplast is a thin " pellicle " that gives a positive reaction for pectin.

In *Isoetes*, as in *Selaginella*, the exospore is the first wall layer to be formed and the mesospore, which Fitting believes to arise *de novo* as in *Selaginella*, is formed somewhat later. The formative events closely parallel those of *Selaginella* and as the megaspore increases in size the mesospore separates from the exospore, remaining attached at the proximal pole. In some species, mesospore separation does not take place at all or only to a very limited extent. Endospore deposition occurs when the megaspores have almost attained their full size.

One further point is worth recording. Fitting (following Tschistiakoff) recommends that the term " epispore " should be applied to the membrane or membranes formed from the " Epiplasma " (the tapetum ?) and the term perispore to that of those formed from the special mother cell wall. Following these definitions, the outer silicified layer of *Isoetes* megaspore walls, which Fitting describes as originating from the inner layers of the special wall, would be a perispore.

Ultra-thin sections for electron microscopy which show exine structure in *Isoetes* megaspores have not so far been obtained. Thin sections for examination by optical microscopy are much easier to cut and form the basis of the following description.

Pl. 5, figs. 7, 8 are anoptral contrast photographs of thin sections of megaspores of *Isoetes humilior* F. Müll. ex A. Braun that were soaked in hydrofluoric acid to remove the siliceous material from the exine and acetolyzed prior to embedding in epoxy resin. The resolution attainable by this method of examination is sufficient to confirm Fitting's (1900) observation that the exospore (ektexine) is composed of small " crystalline " rods arranged parallel to the surface of the spore. Histochemi-

cal tests on megaspores not subjected to hydrofluoric acid treatment show, however, that the exine elements or rods are composed of sporopollenin.

Although the arrangement of the sporopollenin elements forming the ektexine in the megaspores of this genus somewhat resembles that seen in *Selaginella*, there are some marked differences. Comparison of the micrographs of *Selaginella* (Pl. 4, figs. 1, 2 ; Pl. 5, fig. 1) with those of *Isoetes* show that in the latter the ramifying rodlets of the ektexine are thinner and much less densely packed than in *Selaginella*, the general effect being that the *Isoetes* exine appears to have a more "open" texture. A second difference is that an inner lamellated endexine was not detected in the megaspores of *Isoetes*.

In a number of megaspores of *Isoetes* that were examined as both sections and entire objects the inner part of the ektexine is seen to be separated from the remainder (Pl. 5, fig. 8 ; Pl. 6, fig. 1) as described by Fitting. The separation would seem to be quite unrelated to the age of the spores, and evidently occurs along a plane of weakness in the exine. It is never complete around the full circumference of the megaspore and attachment is always maintained at some point, usually the proximal pole.

Microspores of Isoetes. Electron micrographs of *Isoetes echinospora* Dur. microspores show that the exine consists of two layers, the outer is lamellated and the inner homogeneous (Pl. 6, figs. 2, 3). The lamellae of the outer layer appear to anastomose in some sections and the layer then appears structurally not unlike the ektexine of the megaspores. The arrangement of these subdivisions is particularly interesting because the microspores of this genus are the only spores so far examined where the outer layer of the exine is lamellated. Nilsson & Praglowski (1963) however, call this layer the perine and do not, therefore, regard it as part of the exine *sensu stricto*. This interpretation, together with the question of just what does and what does not constitute a perine, is discussed later.

The electron micrographs and phase contrast pictures show that in *Isoetes*, although there are some structural differences between the exine of the microspores and that of the megaspores, they are not so marked as they are in *Selaginella*.

The phase contrast pictures of sections of acetolyzed microspores from a single sporangium of *Isoetes echinospora* (Pl. 7, figs. 1–8) show that the inner layer of the exine is invariably detached from the outer, but the occasional spore exhibiting complete unity between these two layers, presumably the normal condition (Pl. 7, fig. 1), serves to suggest that the separation is either an unusual feature of development or an artifact induced by fixation. Variations in the extent to which the layers have separated are shown. In some of the microspores the inner layer is attached only at the proximal pole (Pl. 7, figs. 4, 5) and in others only at the distal pole (Pl. 7, figs. 2, 3). One example shows a condition where the inner membrane is attached laterally and is completely free at both the proximal and distal poles (Pl. 7, fig. 8) and others where the inner membrane is completely free from the outer around its entire circumference (Pl. 7, figs. 6, 7). Thus, within a single microsporangium of *Isoetes*, spores can be found that could be classified when dispersed

as either cavate or not so, if the current definition for this structural condition was applied to them (see Dettman 1963, Kremp 1965).

Somewhat similar variations in the arrangement of the wall layers occur in the microspores of *Marsilea* that are described below.

The wall structure of *Laevigatisporites* cf. *glabratus*

Megaspores identified as *Laevigatisporites glabratus* (Zerndt) Potonié & Kremp 1954 were recovered from the Lawrence Shale (Virgillian) of Lone Star Lake, Kansas by digesting the sediment in cold hydrofluoric acid. Megaspores of the *Laevigatisporites* type have been recorded from a number of Carboniferous Sigillarian fructifications (see Chaloner 1953).

Electron micrographs (Pl. 7, fig. 10) and phase contrast photographs (Pl. 6, fig. 5) show that the entire thickness of the exine is composed of the familiar ramifying units of sporopollenin. The presence of an inner wall layer (endexine) bordering the spore lumen was not detected, but Schopf (1941) has found two wall layers in the microspores of *Mazocarpon oedipternum* (a Sigillarian cone with *L. glabratus* megaspores).

The spores of *Marsilea* and *Regnellidium*

Megaspores of Marsilea. Feller (1953) and Boterberg (1956) have both described the structure and development of the megaspores and microspores in *Marsilea*—Feller in *Marsilea hirsuta* and Boterberg in *Marsilea diffusa*. Both authors compare their observations with those published by Meunier (1888) on the structure of the sporocarp and spores of *Pilularia globulifera* and Boterberg compares his with those of Bonnet (1955).

Although Feller's and Boterberg's respective accounts of spore ontogeny in *Marsilea* differ in some relatively minor details they both conclude that the megaspore in the species they have investigated is composed of an inner endospore and an outer exospore. Three outer, rather unique gelatinous layers surround the exospore, but these are not considered in this account.

According to these two authors the endospore is the first megaspore wall layer to develop and when fully differentiated in the mature megaspore it consists of two layers. From the evidence presented by these authors the inner endospore probably corresponds to the intine of an angiosperm pollen wall; the outer layer is sudanophilic. In young megaspores the endospore is often found detached from the remainder of the wall.

The structure of the exospore of mature *Marsilea* megaspores is very complex. Immediately overlying the endospore is the inner division of the exospore which Feller (1953) and Boterberg (1956) call the "reticulated layer". This layer is continuous around most of the megaspore but gradually thins and disappears in the region of the germinal sutures. The "prismatic layer" forms the outer division of the exospore and also the outer layer of the megaspore exine *sensu stricto*. Both authors give a precise and detailed account of the development of the "prismatic

layer " in *Marsilea* and the reader is directed to their publications for this information. In mature megaspores the " prismatic layer " consists of a basement membrane upon which is arranged a number of radially-directed elements that, under high magnifications, can be resolved as small prismatic chambers. According to Boterberg (1956) the chambers are open to the outside and during development are penetrated by digitations of the tapetal periplasmodium. Towards the spore apex the " prismatic layer " decreases in thickness and is not developed in the region of the germinal sutures.

The value of Ehrlich's haematoxylin and Orange G, Congo red, Fast green and Analine blue used by Feller (1953) and Boterberg (1956) in detecting the first appearance and subsequent deposition of sporopollenin in the wall layers is very limited. For example, Boterberg (1956) states that in mature or almost mature megaspores the " reticulated layer " is stained by Fast green, Analine blue, Congo red and Orange G. None of these stains is specific or even selective for lipoidal substances. The electron micrographs published here (Pl. 8, fig. 2) show that the " reticulated layer " in acetolyzed megaspores of *Marsilea quadrifolia* L. is composed of anastomosing rodlets of acetolysis-resistant material, and that the spaces between could have been occupied by some material that did not survive acetolysis. It is not impossible that the stains employed by Feller and by Boterberg are in fact colouring some interstitial substance between the sporopollenin elements rather than the exine material proper, even assuming that this had in fact been formed.

It is a well known fact that in the megasporangium of *Marsilea* only one fertile spore is produced. Although all the sporocytes in the megasporangium undergo meiosis, only a single spore from one of the tetrads so formed develops to maturity ; the remainder abort. Both Feller (1953) and Boterberg (1956) give an account of this very interesting phenomenon. They find that the three abortive spores that remain attached to the developing megaspore are surrounded by a membrane, the endospore, which is formed at the same time as the endospore of the fertile megaspore. Wall layers external to the endospore are not formed around the abortive members of the tetrad.

Acetolyzed megasporangia from mature sporocarps of *M. quadrifolia* and *Marsilea drummondii* A. Br. yield megaspores which often bear small acetolysis-resistant bodies attached at the apex (Pl. 9, figs. 1, 2). This situation is very reminiscent of that seen in the Devonian and Carboniferous megaspore tetrads of *Cystosporites*. Thin sections through the apical region of acetolyzed *M. drummondii* megaspores examined by phase contrast illumination (Pl. 9, fig. 2) show that the abortive spores are represented by small, irregularly-shaped masses of sporopollenin with small hollow vesicles, and appear very much as shown in the illustration of Boterberg (1956). There is none of the complex structural organization seen in the exine of the fertile megaspores.

Electron micrographs of ultra-thin sections through the exine of a fertile megaspore of *M. quadrifolia* illustrate that the exine stratification detected by Feller and by Boterberg in the megaspores of *M. hirsuta* and *M. diffusa* is the same in this species.

The inner layer of the exine (endexine) which is equivalent to the outer endospore of Feller and Boterberg is composed of sporopollenin which exhibits a granular structure in sections stained with potassium permanganate. On the inner border of this layer is a zone which is slightly more electron opaque than the remainder (Pl. 8, fig. 2).

The " reticulated layer " of the French authors is seen in electron mircographs to be composed, as mentioned above, of anastomosing rodlets of sporopollenin (Pl. 8, figs. 2, 3). The rodlets are more or less circular in cross-section and are surrounded by clear spaces that in the living spore were presumably filled with some interstitial substance. At the junction of the " reticulated layer " and the subjacent endexine the rodlets of the former are seen to arise directly from the somewhat irregular surface of the latter. There is thus structural continuity between the endexine and the innermost layer of the ektexine.

The most clear indication of the structural organization of the " prismatic layer " is obtained from thin sections examined by phase contrast illumination (Pl. 8, fig. 4). The outer terminations of the radially-directed chambers are seen to be covered by a thin, continuous, acetolysis-proof membrane. A membranous cover closing the prismatic chambers was not detected in *M. hirsuta* (Feller 1953) nor in *M. diffusa* (Boterberg 1956). The radial walls of the chambers are perforated with small, irregularly-shaped apertures which in sections of non-acetolyzed material are somewhat obscured by aggregations of small phase-dark granules adhering to the walls. There is some visual evidence to suggest that at their innermost limit the walls of the prismatic chambers divide, although the junction between the " prismatic layer " and the " reticulated layer " appears quite distinct under the optical microscope (Pl. 8, fig. 4). Electron micrographs of this region show, however, that the elements of the " prismatic layer " are in places continuous with those of the " reticulated layer " (Pl. 8, fig. 3). The fine structural continuity between the three layers comprising the megaspore exine of *M. quadrifolia* suggests perhaps that sporopollenin deposition in the megasporangium is a continuous process. The implication here is that the very marked stratification in the megaspore wall of this genus does not necessarily mean that waves or cycles of sporopollenin deposition are separated by intervals when the mechanism, or mechanisms, responsible for wall formation is inactive.

Microspores of Marsilea. Electron micrographs of radial sections through acetolyzed spores from the microsporangia of *M. quadrifolia* and *M. drummondii* clearly show the so-called " striated " or " prismatic " layer of the exine detected by Feller (1953) and Boterberg (1956). Tangential sections through this layer reveal, however, that the striations are in fact a system of more or less radially-aligned tubes (Pl. 9, fig. 5) that are closed at their apices, but interconnected at their bases (Pl. 9, fig. 4). The inner exine layer (endospore of Feller and Boterberg) appears to have a finely granular ultrastructure.

Both these authors in their respective accounts of microsporogenesis in *Marsilea* show that the microsporangia contain; in addition to the normal and presumably fertile microspores, a variable number of abortive spores. Perhaps the most inter-

esting of these from a morphological point of view are the pseudospores. Pseudospores are described as anucleate, aplasmic spore-like inclusions that develop within small vacuoles in the tapetal periplasmodium. Their formation begins with droplets, which Bonnet (1955) identifies as tannoids but which Feller's evidence would suggest are sporopollenin, becoming surrounded by a thin, sudanophilic membrane. As development proceeds the surrounding membrane assumes an appearance very reminiscent of the outer membrane of the microspores. The final stage of pseudospore development is marked by the formation of one or more gelatinous layers on the outside of the " striated " layer. Feller (1953) recognizes four types of pseudospores that are separable on the form of the central inclusion, and there are structural intermediates between the four types. None of these contains a nucleus or cytoplasm and they do not appear to form in tetrads.

" Microspores volumineuses " are a second type of abnormal spore described by Feller (1953) and Boterberg (1956) but these, unlike the pseudospores, are formed from the archesporium. Their formation is due to a blockage in meiosis at or before metaphase II (Boterberg 1956), with the result that individual haploid microspores are not formed. " Microspores volumineuses " are, therefore, diploid, but the French authors find that exine development proceeds more or less normally and eventually the diploid spores are surrounded by a sudanophilic wall consisting of a thick endospore and a thinner " striated " overlying membrane. In the space between these two layers are small droplets of various shapes and sizes.

Acetolyzed microsporangia of *M. quadrifolia* and *M. drummondii* have been found to contain a large number of spores which from their structure when examined as entire objects could be equated with one or other of Feller's (1953) four types of pseudospores (Pl. 10, fig. 1). Examination of thin sections of these structures with anoptral contrast illumination suggests that the situation in my material cannot be fully explained on the basis of the interpretation of pseudospore formation given by Feller.

The sections show that each pseudospore is surrounded by an outer exine layer composed of radially-aligned tubes which seem to be exactly similar to the outer membrane of the normal, fertile microspores (Pl. 10, figs. 3–8). There is, however, considerable variation in the extent to which the inner wall layer is developed. In some of the smaller pseudospores it is not present as a distinct membrane but is represented merely by small, discrete, phase-white droplets of acetolysis-resistant material adhering to the inside of the external layer. The partial or nearly complete coalescence of these droplets in some specimens appears to result in a structure not unlike a membrane (Pl. 10, fig. 4). In somewhat larger pseudospores the inner layer can be seen as either a small central body having no structural connexion with the outer layer (Pl. 10, fig. 5) or as a much folded membrane that has contracted away from the outer layer (Pl. 10, figs. 7, 8). In both these forms (some specimens of which may be " microspores volumineuses ") small acetolysis-resistant, phase-white droplets are attached to the inner surface of the outer membrane. Pl. 10, fig. 6 shows a section of a spore the wall of which seems to differ from the normal condition (Pl. 10, fig. 3) only in the slight thickening of the inner layer and the

partial contraction of this layer away from the external layer of the exine.

It should perhaps be emphasized here that the abnormal spores or pseudospores constitute the greater part of the contents of the microsporangium of *M. quadrifolia* and *M. drummondii*. The sections of these structures show that in all of them, no matter how contorted the inner membrane may be, a central lumen is always present. Cytoplasmic contents could not, however, be detected in the sections of non-acetolyzed material treated with appropriate nuclear and cytoplasmic stains. Nevertheless, the presence of a central lumen would seem to suggest, although not prove, that at some time they did possess cytoplasmic contents.

The evidence available from the sections indicates that the abnormal spores have to some extent followed the same developmental pattern as the fertile spores, but that at some stage during their development the process of wall formation has deviated from the normal. There are several relevant factors to be considered in this connexion. Firstly, both the normal and abnormal spores occur together in the same sporangium. Secondly, in all cases it seems to be only the inner exine layer of the abnormal spores that is malformed ; the outer membrane is precisely the same as in the normal spores. Electron micrographs confirm the optical observations on this point. Finally, the outer wall layer often assumes a shape that is quite independent of that assumed by the inner membrane.

Feller (1953) and Boterberg (1956) have found that in the development of the normal microspores the endospore is the first layer formed around the protoplast. If it can be assumed that this is also true for the abnormal spores then the endospore in these must have been formed around either a normal protoplast which subsequently degenerated or a protoplast that was degenerate from the start. The fact that the outer membrane in these spores is completely normal and does not necessarily follow the contours of the endospore would seem to lend support to the first alternative. However, there are points which still remain to be explained. Whilst the degeneration and subsequent contraction of the protoplast could be responsible for the collapse of the endospore it is difficult to see how it could account for the increased thickness of this layer in the abortive spores. The presence of acetolysis-proof droplets which may represent the inner layer in some spores and those that occur between the two wall layers in others also needs to be explained.

It is difficult to reconcile Feller's (1953) interpretation of the origin and mode of development of aplasmic, anucleate pseudospores with the level of structural organization revealed in the abortive spores described above. Heslop-Harrison (1963*a*, *b*) has shown that the protoplast of pollen grains is involved in the establishment of the exine pattern and it is of more than local interest to establish precisely how the spore wall is formed in the abortive microspores of *Marsilea*, and at what stage during the process the spore protoplast begins to degenerate.

One further point should be mentioned here. Feller (1953) and Boterberg (1956) refer to small granules of material, which chemically resemble the exine substance, occurring in blebs that arise from the tapetal periplasmodium adjacent to the developing microspores. Feller, who follows their development, believes that these granules contribute towards the formation of the microspore exine. The granules

may well be identical with the Ubisch bodies described by Rowley (1963) or the tapetal plaques detected by Heslop-Harrison (1962, 1963*b*) in the pollen sacs of angiosperms.

Shattuck (1910) described how the degree of spore abortion in the sporangia of *Marsilea* can be controlled by varying the external conditions under which the plants are growing. He shows that there is some correlation between the time during spore development at which the stimulus (a change in temperature or light intensity) is applied and the resultant fertility of the spores. The critical period in this respect would seem to be from the time the mother cells enter synapsis until the tetrads are well formed. Shattuck advances the suggestion that a mechanism of this sort could have been responsible for the origin of heterospory in *Marsilea* and possibly for its origin in all heterosporous plants. By varying the external conditions and reducing the number of developing sporocarps on a plant Shattuck was able to induce the formation of large spores or " megaspores " in microsporangia and small spores or " microspores " in megasporangia. Shattuck is not unaware of the very significant differences between heterospory and heterothally and he attempted, without success, to germinate the experimentally produced " megaspores " and " microspores ". Thus, the only reliable criterion that would test the validity of his claims, the production of megagametophytes and microgametophytes, could not be applied. Nevertheless, the possible significance of his observations cannot be ignored.

The megaspores of *Marsilea* have, unquestionably, the most complex exine structure of any megaspores from either living or fossil plants so far investigated. It is considerably more complex than that seen in the megaspores of the lycopods *Selaginella* and *Isoetes*. Although the exine structure of the microspores of *Marsilea* appears superficially similar to that of the megaspores, ultra-thin sections show that there is a large measure of difference between the two.

Megaspore of Regnellidium. Chrysler & Johnson (1939) describe the wall of the mature megaspore of *Regnellidium diphyllum* Lindm. as consisting of two principal layers, the endospore and the epispore ; the latter being subdivided into three sublayers. According to their interpretation the endospore lies next to the spore protoplast and is continuous under the apical papilla. The homogeneous inner layer of the epispore is sudanophilic, lies closely applied to the endospore and is not continuous under the papilla. The thickest layer of the megaspore wall is formed by the middle layer of the epispore, the prismatic layer. This is composed of hollow radiating chambers or prisms the outer ends of which are closed by a membranous cap. The prismatic layer is also sudanophilic. The outer layer of the epispore is termed the papillate layer but since microchemical tests show it to be composed of cellulose it will not be considered further. Finally, a thick layer of mucilagenous material was detected between the papillate layer and the sporangium wall, but the authors do not regard this as part of the megaspore wall.

Although Chrysler & Johnson (1939) do not record the presence of a layer internal to the endospore in the megaspores of *Regnellidium* they do illustrate sections of spores which clearly show a membrane in this position and which they regard as a

layer of the cytoplasm. This layer is continuous over the floor of the papilla and appropriate histochemical tests would determine whether it is analogous to the intine of angiosperm pollen.

The megaspore wall of *Regnellidium* has not so far been examined in the electron microscope, but sections of acetolyzed material examined by phase contrast microscopy show that the exine stratification, although somewhat similar to that of *Marsilea* megaspores, differs sufficiently to warrant description.

Under phase contrast the inner layer of the megaspore wall (endexine or endospore of Chrysler & Johnson) appears to be homogeneous (Pl. 10, figs. 9, 10). The layer immediately overlying the endexine (inner layer of the epispore of Chrysler & Johnson) is not homogeneous, but structurally resembles the " reticulated layer " of *Marsilea* megaspores. In *Regnellidium*, however, the " reticulated layer " is thicker than in *Marsilea* and subdivided into two very distinct zones, the outer one of which has a coarser structure than the inner (Pl. 10, fig. 10).

In the megaspores of *Marsilea* the radially-arranged hollow chambers or prisms are covered by a continuous membrane which passes without interruption across their outer surfaces. In *Regnellidium* the situation is somewhat different. The membrane terminating each chamber is not continuous and the result, as Chrysler & Johnson (1939) point out, is that the outer surface of the layer appears papillate (Pl. 10, fig. 9). Enclosed at the outer end of each of the chambers is an accumulation of acetolysis-resistant material in the form of small phase-dark granules.

The microspore of *R. diphyllum* is, according to Chrysler & Johnson, a small edition of the megaspore. Two wall layers can be detected, an endospore and an outer prismatic layer. The authors observed several cases where the prismatic layer surrounded an entire tetrad of microspores or, in some cases, possibly even two tetrads.

SPORES OF LIVING AND FOSSIL HOMOSPOROUS PTERIDOPHYTES

The spores of three genera of living homosporous pteridophytes (*Lycopodium*, *Psilotum* and *Asplenium*) have been examined to determine how the exine of these spores compare with the microspores of living and fossil heterosporous pteridophytes. In addition, one fossil dispersed spore from the Devonian (*Archaeotriletes*), the parent plant of which is unknown but is assumed to have been homosporous, has been sectioned.

Spores of *Lycopodium*

The electron micrographs of acetolyzed and chlorinated *Lycopodium clavatum* spore walls published by Afzelius, Erdtman & Sjöstrand (1954) show that the exine is divided into an outer lamellated and an inner granular layer. Afzelius (1956) shows how the fine structure of the spore exine can be destroyed by over-chlorination or treatment with chromic acid.

Acetolyzed spores of *Lycopodium selago* L. (Pl. 10, fig. 11 ; Pl. 11, fig. 1) do not display the striking lamellation detected in the spores of *L. clavatum*, and the exine

appears to be more or less granular to faintly fibrous. On the proximal side of the spores a discontinuous granular zone underlies the outer wall layer and this is ultrastructurally similar to the granular layer illustrated by Afzelius, Erdtman is Sjöstrand (1954). The rather irregular occurrence of the layer in *L. selago*, however, suggests that it is produced by the breakdown of the outer wall layer and should not be regarded as a separate stratum of the exine in these spores.

The fine structure of the spore wall in *L. selago* differs sufficiently from that detected in *L. clavatum* to justify an investigation to determine the full range of variation within the genus.

Spores of *Psilotum*

An acetolyzed exine of a *Psilotum nudum* Griseb. spore section stained with lead hydroxide is shown on Pl. 11, fig. 3. It can be seen that the wall is weakly stratified. A finely granular layer surrounds the spore lumen and to the outside is a somewhat thicker fibrous stratum. The undulating outer and inner margins of the wall have borders of rather higher electron density than the rest of the exine.

Spores of *Asplenium*

The spores of the polypodiaceous fern genus *Asplenium* have, according to Bower (1923) and Sorsa (1964) a distinct perispore or perine. The perispore is regarded as being the last layer formed by the tapetum. In the majority of species it is said to be represented by a thin, loosely-attached, often highly ornamented membrane (see, for example, Sorsa 1964). Erdtman (1952) does not consider the perispore as part of the exine in the strict sense, but rather as an extra-exinous layer of the sporoderm.

Electron micrographs of ultra-thin sections of acetolyzed spores of *Asplenium adiantum-nigrum* L. show that the wall is stratified into three distinct layers. The outer wall layer, or perispore, is very electron opaque. Stratification is clearly detectable within the perispore itself and is seen as a series of electron-dense membranes (two outer and two inner) enclosing two granular interstitial layers (Pl. 12, figs. 1, 2). The electron micrographs show that only the two outer perispore membranes and the outer granular layer that they enclose contribute to the formation of the elements which form the ornamentation of the spore. The two inner perispore membranes and the inner granular layer continue uninterrupted beneath the projections, and as a result they have a hollow central cavity.

Between the perispore and the inner layer of the spore wall is a space, the presence of which presumably accounts for the fact that the perispore is easily detached. The inner layer of the wall would constitute the whole of the exine *sensu stricto* according to Erdtman's (1952) generally accepted terminology for perinate spores. This layer in *A. adiantum-nigrum* is structurally homogeneous in most sections, but appears faintly granular in a few (Pl. 12, figs. 1, 2). The inner and outer edges of the layer are bordered by two very thin slightly more electron-opaque zones.

The exine fine structure of the spores of *A. adiantum-nigrum* is quite unlike the

microspores of *Selaginella*, *Isoetes* and *Marsilea* or the spores of *Lycopodium* and *Psilotum*. By definition the perine of the *Asplenium* spores is a wall layer which has no homologue in these species, but this interpretation can be questioned and is discussed later. The fact that the inner wall layer or exine of the *Asplenium* spores is homogeneous and completely undifferentiated is not altogether a surprising discovery. Any discussion as to its possible significance would, however, be almost valueless without substantially more information on the range of ultrastructural variation in fern spores, particularly in the phylogenetically more primitive genera.

The wall structure of *Archaeotriletes*

The fossil spores provisionally referred to this rather broad genus were isolated with 40% hydrofluoric acid from a sandstone of Upper Devonian age from Scaumenac Bay, Quebec, Canada.

The botanical affinity of the spores is not known, but they possess, in common with a number of other genera of the same age, an ornamentation which consists of appendages that terminate in grapnel-shaped hooks (see Naumova 1953). The exine of *Archaeotriletes* has not been studied as completely as some of the other spores described in this account, and the observations are included as a first assessment which may have to be modified later.

Ultra-thin sections of *Archaeotriletes* show that the outer part of the exine which forms the wall ornamentation is composed of anastomosing rodlets of sporopollenin similar to those seen in the spores of *Archaeopteris*, the megaspores of *Selaginella* and *Isoetes* and the Carboniferous lycopod megaspore *Laevigatisporites*. The impression gained from some sections is that the orientation of the elements changes slightly towards the inside so that the wall appears to be weakly stratified (Pl. 12, fig. 4). So far a layer internal to the spongy layer has not been detected. Lastly, it can be seen that the spines which constitute the ornamentation of the spores are formed by the spongy layer and that they have a hollow central cavity (Pl. 12, fig. 3).

POLLEN OF FOSSIL AND LIVING GYMNOSPERMS

The exine structure of *Schopfipollenites*

Pollen belonging to the genus *Schopfipollenites* Potonié & Kremp 1954 was obtained from the Lawrence Shale, Lone Star Lake, Kansas. The Lawrence Shale is of Pennsylvanian (Virgillian) age.

Schopf (1949) has described pollen of the *Schopfipollenites* type in the medullosan pteridosperm fructifications *Dolerotheca* and *Whittleseya*, and there is no doubt that *Schopfipollenites* is a genus based on pollen. However, because the grains have both a proximal monolete aperture (a feature characteristic of some types of spores) and two distal grooves or sulci (a single sulcus is a feature characteristic of some pollen) Schopf has adopted Renault's term " prepollen " for the fossils. In view of Afzelius's (1956) suggestion that some submicroscopic features are more

primitive than others it was considered of particular interest to examine the wall structure of *Schopfipollenites* by electron microscopy and to compare it with the structure detected in the microspores of the free-sporing vascular cryptogams and the pollen of living gymnospermous seed plants.

The exine of *Schopfipollenites* is divided into two very distinct layers which can be clearly seen with the optical microscope (Schopf 1949). In ultra-thin sections examined with the electron microscope the fine structural details of the two layers is resolved.

The inner exine layer (endexine) is distinctly lamellated (Pl. 13, figs. 2, 3 ; Pl. 14, fig. 1) but in places the lamellation is scarcely detectable doubtless as a result of compression during the fossilization process. In one section (Pl. 13, fig. 3) it appears as though the endexine is itself subdivided into two layers.

The outer layer of the exine (ektexine) is constructed of the familiar anastomosing units of sporopollenin giving the wall a spongy appearance. Structural distortion due to compression is also recognizable in this layer. Continuity exists between the endexine and the ektexine and in some of the micrographs (Pl. 13, fig. 2 ; Pl. 14, fig. 1) it can be seen that the innermost units of the ektexine arise directly from the outer surface of the endexine.

Towards the outer edge of the pollen grain the sporopollenin units of the ektexine are progressively more coalescent and at the periphery are completely fused to form a solid margin. Under the optical microscope entire specimens of *Schopfipollenites* from the Lawrence Shale appear to be minutely punctate (Pl. 13, fig. 1). The electron micrographs show, however, that the solid margin is not interrupted by punctae (Pl. 13, fig. 2). One possible explanation to account for this discrepancy is that the interstitial spaces between the outermost sporopollenin units of the ektexine can be mistaken for punctate surface ornamentation when the pollen is examined by transmitted light.

In the possession of a lamellated endexine and a spongy ektexine the exine of the pteridosperm pollen *Schopfipollenites* very closely resembles that of the spores of *Archaeopteris* and the megaspores of *Selaginella*. This fossil pollen is, in fact, ultrastructurally considerably more " spore-like " than " pollen-like ". This topic will be returned to later.

Pollen of *Encephalartos*

Sections of acetolyzed pollen from the living cycad *Encephalartos villosus* Lem. clearly show that the exine is stratified into three distinct layers.

The proximal surface of the grain is covered by an outer ektexinal layer of homogeneous to faintly fibrous sporopollenin that extends laterally round the sides of the grain and enters the distal sulcus where it rapidly thins and finally disappears (Pl. 15, figs. 1, 2). The inner and outer margins of this layer are interrupted by small cavities or pits (Pl. 14, fig. 2). Immediately underlying the outer ektexine is the middle wall layer or inner part of the ektexine which in thin sections appears to be prismatic. This layer is not well developed on the proximal face of the grain, but becomes thicker towards the sides and is thickest in the distal sulcus where,

with the disappearance of the outer ektexine, it is the outer layer (Pl. 15, figs. 1, 2). In sections that pass obliquely through the inner ektexine it can be seen that the elements forming the layer are a system of radially-aligned closed tube-like processes. The outer extremities of the elements are connected to the inner surface of the outer ektexine, and the inner extremities to the innermost exine layer. In the distal sulcus, where the outer ektexine is not formed, the elements of the middle layer are terminated by a thin, continuous membrane which passes across their outer ends. This membrane could be considered to represent the outer ektexine in this region.

The endexine in *E. villosus* pollen is a very thin lamellated layer (Pl. 14, fig. 2). Not infrequently, the endexine and the inner ektexine become detached from the outer ektexine at the sides of the pollen grain so that they curve into the pollen lumen ; the effect is to make the grain appear saccate (Pl. 15, fig. 1).

Afzelius (1956) and Ueno (1960) have published electron micrographs showing the exine structure in the pollen of *Cycas*. Afzelius finds that the pollen exine of *Cycas revoluta* is composed of two layers, the inner layer being lamellated and the outer one being composed of branched bacula-like elements. Ueno, on the other hand, detected three layers in the pollen exine of *Cycas taiwaniana* which appear to correspond to the three layers in the pollen of *E. villosus*, although there are some small differences. The stratification in the pollen wall of *Ceratozamia mexicana* described by Larson (1964) closely resembles that found in *Cycas*. In *C. mexicana* also the innermost layer of the exine is lamellated.

Compared with the Carboniferous pteridosperm prepollen *Schopfipollenites* the exine stratification revealed in the pollen of living cycads is quite complex. One notable feature shown by Ueno's (1960) illustrations of *C. taiwaniana* and those of *E. villosus* illustrated here (Pl. 14, fig. 2 ; Pl. 15) is that the exine of cycad pollen grains is thinner in the distal sulcus or germinal furrow than in the rest of the wall. But this is not so in *Schopfipollenites*. Perhaps the distal sulci in the pteridosperm prepollen are not true germinal furrows but merely a harmomegathic region and germination may have occurred through the proximal suture, as in the spores of free-sporing pteridophytes.

Pollen of *Taxus*

In glutaraldehyde-fixed pollen of *Taxus baccata* L., post-fixed in osmium tetroxide, almost the entire thickness of the exine is conspicuously lamellated (Pl. 16, figs. 2, 3). On the outside of the lamellated layer is a zone rather than a uniform layer consisting of small, electron-dense droplets of exine material. Erdtman, Berglund & Praglowski (1961) call these structures orbicules, and they undoubtedly account for the pollen wall ornamentation. The orbicules vary somewhat in size and many bear small conical projections which radiate from their surfaces. The arrangement of these bodies on the surface of the lamellated layer seems to be quite haphazard. Some lie directly on the lamellated layer and in close contact with adjacent orbicules so that there is a suggestion of partial coalescence between them. Others lie completely free and some distance above the lamellated layer. At high magnifications a substance with a granular fine structure can be detected between the orbicules

and the lamellated layer. This substance appears to bind the innermost orbicules to the subtending layer and in some cases to cement the orbicules together (Pl. 16, fig. 2).

Between the lamellated layer and the pollen cytoplasm is a fairly thick, electron-transparent intine which is structureless in material fixed in glutaraldehyde and post-fixed in osmium tetroxide.

Ueno (1959) considers the small spinous droplets covering the *Taxus* exine as perine which is united with the outer exine layer or sexine. The sexine is the name applied by Ueno to the granular substance situated between the droplets and the lamellated layer. Certainly, the very scattered distribution and somewhat irregular appearance of the orbicules are very unusual features which perhaps have their nearest homologue in the perispore of the pteridophytes rather than in a true exine layer. Of some significance in this connexion are the small electron-dense droplets which occur in the innermost pollen sac tapetal cells of *Taxus*. These droplets closely resemble those attached to the pollen exine (Pl. 16, fig. 4). Similar droplets also occur between adjacent pollen grains and seemingly lie free in the pollen sac locule. They can be compared with the Ubisch bodies described by Rowley (1963) and the tapetal plaques of Heslop-Harrison (1962, 1963*b*). Sporopollenin droplets of this kind are not confined to *Taxus* and Ueno (1959) has shown that they occur in a number of other coniferous genera. Afzelius (1956) also draws attention to their presence in *Cephalotaxus*. There is also some apparent structural homology between these structures in the conifers and those that form the outer layer or zone of the spore wall in the moss *Dicranum scoparium* Hedw. described below.

Pollen of *Pinus*

The ultrastructure of the pollen wall of *Pinus* has been described by Mühlethaler (1955), Ueno (1958) and by Ting & Tseng (1965). The electron micrographs of exine structure in *Pinus balfouriana* published by the latter authors show that the inner wall layer (endexine) in this species is granular in composition. Overlying the endexine is a thicker ektexine which they subdivide into a " foot layer " or " limen inferum ", a series of columellae with " depressions " and " funnels " and a tectum. Their pictures show that the ektexine expands to form the sacci of the pollen grain whilst the granular endexine alone remains to form the floor of the sacci. In contrast to this, Ueno (1958) has shown that in *Pinus thunbergii* only the tectum and the columellae contribute to the sacci, and the saccus floor is formed by the " foot layer ". This also seems to be the case in *Pinus nigra* (Mühlethaler 1955).

Sections of *Pinus sylvestris* L. pollen that has been fixed in glutaraldehyde and post-fixed in osmium tetroxide show features which are structurally in accord with those published by Ueno (1958) in *P. thunbergii*. The inner layer of the exine (endexine) in *P. sylvestris* is composed of lamellated sporopollenin (Pl. 17, fig. 4). An ektexine overlies the endexine and consists of a basal layer (" foot layer "), a series of columellae which are sometimes branched (termed the " tigna " by Ting & Tseng 1965) and a tectum. The tectum is folded in places and possibly interrupted by " depressions " and " funnels " similar to those detected in *P. balfouriana*

by Ting & Tseng (Pl. 17, fig. 3). The basal membrane (" foot layer ") of the ektexine appears to be composed of a series of distinct droplets of the exine material which lie in close contact and which, in places, are partly coalescent. However, it seems likely that the droplets are arranged along a thin and continuous membrane which they are obscuring (Pl. 17, fig. 4).

One notable feature is the difference in the construction of the sacci in *P. sylvestris* and in *P. balfouriana*. In the former, unlike the latter, the saccus is composed only of the columellae and the tectum of the ektexine (Pl. 17, fig. 2 ; Pl. 18, fig. 1), the inner layer or " foot layer " has not expanded but remained to form the floor of the saccus precisely as in *P. nigra* (Mühlethaler 1955) and *P. thunbergii* (Ueno 1958).

Another marked difference between the two species is in the fine structure of the endexine. In *P. balfouriana* it is granular, in *P. sylvestris* it is distinctly lamellated. Although Ueno (1958) does not comment directly about the structure of this layer in *P. thunbergii*, judging solely from his drawings it appears to be lamellated.

The structural variation of the endexine in the pollen of *Pinus* could be of taxonomic and possibly of phylogenetic importance, and a full comparative ultrastructural investigation into the wall structure of both living and fossil species would be worthwhile.

Lastly, and merely to record the observation, Ubisch body-like objects can also be detected adjacent to the tapetal cell walls in *Pinus sylvestris* pollen sacs (Pl. 18, fig. 2).

THE MEGASPORE MEMBRANE OF PALAEOZOIC SEEDS AND SEED-LIKE STRUCTURES

It has already been pointed out that the structure of the megaspore membrane in some Carboniferous cordaite and pteridosperm ovules is very different in construction from that of the ovules of living gymnosperms (Pettitt 1966). The observations described below present the evidence in support of this statement.

The megaspore membrane of Carboniferous cordaitalean and pteridosperm ovules

The cordaitalean ovules from which the megaspore membranes used in this study were obtained occur as compression fossils in the Lawrence Shale (Virgillian) of Lone Star Lake, Kansas. The cuticle morphology of the ovules (to be described elsewhere) is very characteristic and leaves no doubt that they are cordaitalean. The pteridosperm megaspore membranes were isolated with hydrofluoric acid from " nucule " casts of *Trigonocarpus* from the British Coal Measures.

Ultra-thin sections of a methacrylate-embedded cordaite megaspore membrane from the Lawrence Shale are shown on Pl. 19, figs. 1–3. It can be seen that the entire thickness of the membrane is composed of a three-dimensional network of sporopollenin elements giving a spongy appearance. At higher magnifications (Pl. 19, fig. 2) the homogeneous structure of the elements is apparent. At the

inner edge of the membrane, in contrast to the outer, the sporopollenin units are fused to form a distinct margin around the lumen (Pl. 19, fig. 3).

The very real structural resemblance of the megaspore membrane of these fossil ovules to the wall structure detected in the megaspores of *Selaginella*, the spores of *Archaeopteris* and the Carboniferous lycopod megaspore *Laevigatisporites* is of considerable interest. A repetition of this structure in the megaspore membrane of *Trigonocarpus* (Pl. 18, fig. 3) the ovule of a Carboniferous pteridosperm and in *Taxospermum*, a petrified cordaitalean ovule (Pl. 18, fig. 4) adds significantly to the statement that structurally the megaspore membranes of the early seed plants more closely resemble the spores of the free-sporing heterosporous pteridophytes than the megaspore membranes of the modern gymnosperms (Pettitt 1966). The phylogenetic significance of this is discussed below.

Megaspores of *Lepidocarpon*

Megaspore tetrads belonging to the genus *Cystosporites* are known to occur in the Carboniferous seed-like structures *Lepidocarpon*, and there is a great deal of evidence to suggest that all lepidocarp seeds contained tetrads of the *Cystosporites* type. Tetrads of *Cystosporites* are characterized by having one large, sac-like, presumably fertile spore and three, smaller, presumably abortive spores attached at the apex of the large one. It is quite probable that most species included in the genus do represent lepidocarp seed megaspores. Only the wall structure of the large fertile spore is described in this account.

Megaspore tetrads referable to *Cystosporites giganteus* (Zerndt) Schopf 1938 were isolated from a Lower Carboniferous coal from Fife in Scotland by digesting the matrix in dilute nitric acid. Chaloner (1952) has shown that megaspore tetrads of *C. giganteus* occur inside the lepidocarp seed *Lepidocarpon waltoni*.

The wall of the large spore of *C. giganteus* exhibits a very characteristic mesh-like or fibrous texture when examined in surface view with the light microscope (see Chaloner & Pettitt 1964). In contrast to this the walls of the three abortive spores appear to be homogeneous or faintly granular when examined in this way.

Examination of sections of the fertile spore by electron microscopy clearly reveals the arrangement and fine structure of the fibrils that compose the outer wall layer. It can be seen (Pl. 20, figs. 1, 2) that the elements form a lose-textured stratum. The electron micrographs show that there are two distinct sizes of fibrils. The larger ones are themselves fibrillar in structure, whilst the smaller ones are homogeneous and more or less circular in cross section. There is evidence from electron microscopy of sections and from phase contrast studies of the surface which suggests that the two types of fibrils are interconnected or that the large fibrous type branch and give rise to the smaller variety.

The inner layer of the *Cystosporites* exine is very thin and is composed of homogeneous sporopollenin (Pl. 20, figs 1, 2).

In his description of *Cystosporites* Schopf (1938) draws particular attention to the fibrous character of the fertile spore exine. He supposes that this type of structure is correlated with the passage of food reserves from the parent plant into the mega-

spore whilst it is enclosed in the sporangium and after the exine was formed. This interpretation would obviously not be applicable to those species of the genus, for example, *Cystosporites verrucosus* and *C. devonicus* (see Chaloner & Pettitt 1964) in which the exine is not fibrous but more compact and homogeneous. The spongy texture of the megaspore wall of *Laevigatisporites*, a sigillarian megaspore described earlier, is not without significance in this connexion. *Sigillaria* is a free-sporing heterosporous plant which had, presumably, an endosporic gametophyte that developed in much the same way as the present day *Selaginella* or *Isoetes*. Whilst the possible physiological significance of a porous megaspore exine surrounding the female gametophyte of *Cystosporites* can be appreciated, that of an equally porous membrane surrounding the gametophytes of *Selaginella*, *Isoetes* and *Laevigatisporites* is not so readily apparent.

The exine structure of *Didymosporites*

The inclusion of an account of the exine of the fossil megaspore tetrads *Didymosporites* under the general heading of seeds and seed-like structures is not meant to imply that these tetrads are known to occur in seeds or even seed-like organs. However, it has been suggested by some authors (see Andrews 1961) that the Lower Carboniferous fossil *Bensonites*, the parent sporangium of *Didymosporites* (Chaloner 1958), may represent a morphological stage intermediate between heterospory and the seed habit. For this reason, as well as that of convenience, the account has been included at this point.

Tetrads of *Didymosporites* are composed of two large, thin-walled, presumably fertile spores and two minute, presumably abortive ones that are situated at the junction of the two large spores (Pl. 21, fig. 4). Chaloner (1958) has isolated *Didymosporites* tetrads from the megasporangium (*Bensonites fusiformis*) of the Lower Carboniferous coenopterid *Stauropteris burntislandica*. He also obtained tetrads of *Didymosporites* (*D. scotti*) from a Lower Carboniferous (Dinantian) coal outcropping east of Pittenweem Harbour in Fife, Scotland and it was from this material that the megaspore tetrads described below were obtained.

The exine of the large, presumably fertile spores of *D. scotti*, when examined as thin sections with the electron microscope, are completely homogeneous and show no detectable stratification (Pl. 21, fig. 1). Anoptral contrast pictures of sections through the entire tetrad (Pl. 21, figs. 2, 3) show that the structural homogeneity extends throughout the fertile spore. The exines of the abortive spores are also homogeneous. A thin, structureless membrane surrounds each tetrad, and this presumably represents, as Chaloner (1958) suggests, a remnant of the tapetum.

IV. GENERAL DISCUSSION

The foregoing descriptions of the structure of the spore and pollen grain walls as revealed by the electron microscope show that there is a considerable amount of ultrastructural variation in the exines of the various genera and the question

arises as to whether the observed structural variations have any phylogenetic significance, and to what, if anything, they can be attributed.

In their work on the structure of the spores of the Musci, McClymont & Larson (1964) find that the wall stratification in all the spores that they examined was basically similar. They also show that the exine of moss spores is rather more simple in construction than that of the vascular cryptogams described above. In the mosses they detected two exine layers, the inner of which is very thin and featureless. The outer exine layer is electron-opaque, acetolysis-resistant and appears to be homogeneous. This layer, according to the authors, corresponds to that which Afzelius (1957) has called the exine in *Funaria* spores. In the spores of some genera (e.g. *Physcosmitrium* and *Encalyptra*) this layer does not participate in the formation of the wall ornamentation, whilst in others (e.g. *Polytrichum* and *Astonum*) it forms either the entire sculptural element or only the base. In the latter case it seems that the major part of the sculptural element is composed of a third layer which McClymont & Larson call the perine. It is also suggested that the perine forms the entire sculptural element in those spores where the exine takes no part in the formation of the wall ornamentation (e.g. *Physcosmitrium* and *Encalyptra*).

Electron micrographs of acetolyzed spores of the moss *Dicranum scoparium* are shown on Pl. 20, figs. 3, 4. In these the exine is composed of a homogeneous to faintly granular layer which bears on its outer surface a number of irregularly arranged and variously shaped droplets that represent the wall ornamentation. These elements closely resemble those which McClymont & Larson (1964) refer to as perine. Low magnification pictures of sections through an acetolyzed capsule of *Dicranum* (Pl. 20, fig. 4) show that the droplets are not only arranged on the spore exines but also randomly distributed in the spaces between the spores and are concentrated in a narrow zone adjacent to the remnants of the capsule wall. The occurrence of the droplets adjacent to the capsule wall strongly suggests that they were in some way associated with the tapetal tissue which has, of course, been destroyed by acetolysis. If these droplets do in fact originate from the tapetal cells, then McClymont & Larson's suggestion that they are perinous (*sensu* Erdtman 1952) would be strictly correct. There is an unescapable resemblance in the arrangement and occurrence of the droplets forming the spore ornamentation in the mosses and the droplets associated with the pollen wall of *Taxus baccata* described earlier (p. 245) and which Ueno (1959) regards as being a perine.

Before continuing there is perhaps some justification for discussing the precise interpretation of the perine. The term perine (or one of its synonyms) has been applied to a variety of exinous or extra-exinous layers of the spore or pollen wall with, seemingly, very little concern as to whether the layer in question is truly perinous. Arising from this is a more important issue ; whether all the layers to which the term has been applied are homologous.

Erdtman (1952) regards the perine as an outer, extra-exinous layer of the spore wall of certain mosses and ferns which is formed by the activity of a tapetal plasmodium with " perinogeneous properties ". He points out that it is virtually

impossible to determine whether a particular layer is exinous or perinous without undertaking a study of the development of the spores.

Harris (1955) also discusses the origin of the perispore or perine and suggests that if the ornamentation of spores arises as a result of the periplasmodial substance gelling on the outer surface of the spore the layer thus formed is the perispore. This definition is essentially the same as that proposed by Bower (1923) and it would seem to restrict the use of the term perine to the spores from plants with a plasmodial tapetum. Rowley's (1963) observation that Ubisch bodies (tapetal plaques, tapetal droplets or orbicules) are apparently only formed in plants with a secretion tapetal system is very relevant in this connexion.

Bower (1923) considers that the most striking development of the perispore is in the Salviniaceae, and regards the massulae in the microsporangium and the glochidia and "swimming organ" of the megasporangia of *Azolla* as perispores with a specialized function. Eames (1936) on the other hand, restricts the term to the meshed structure formed around the megaspore by the fourth massula. Arnold (1955) has shown that this structure survives in fossils.

In the mature microsporangia of *Salvinia* the spores are surrounded by an amorphous mass of vacuolate, sudanophilic, acetolysis-resistant material (Pl. 21, fig. 5) which is formed from the tapetal periplasmodium. Eames (1936) calls this the perispore and, in the sense that this term is defined by Erdtman (1952) and Harris (1955) his usage is correct. A very similar vacuolate mass also occurs in the megasporangium of *Salvinia*.

Fitting (1900), as mentioned before, recommends that the term perispore should be applied only to wall layers that are formed from the special mother cell wall and the term epispore to those formed from the tapetum.

In the spores of *Asplenium adiantum-nigrum*, however, the outer layer of the wall or so-called perispore (see Nilsson & Praglowski 1963) appears, ultrastructurally, to resemble a true exine layer. It is difficult to believe that the perispore in *Asplenium* or in *Isoetes* microspores for that matter, originates directly from a periplasmodium by the process of gelling suggested by Harris (1955).

There is clearly a need for a full comparative developmental investigation into the formation of these wall layers. The correct interpretation can, as Erdtman has said, only be obtained from such a study. The results might well provide an explanation for the presence of the tapetal droplets in the pollen sacs of some conifers and in the capsules of mosses. It would surely clarify the situation with regard to the homology, if it exists, between the structurally distinct perispore in the Salviniaceae, the Polypodiaceae and the conifers and mosses. It might even result in a much-needed, clear and authenticated terminology!

Before leaving the question of tapetal derivatives, one further point is of particular relevance to the present study. It has already been shown (Pettitt 1966) that in certain gymnospermous ovules the structure which is called the megaspore membrane is, in fact, formed by the degeneration of the tapetum to form a thick, acetolysis-resistant membrane. The homologue of this tapetal membrane in the sporangia of free-sporing plants with a plasmodial tapetum would possibly be the perispore.

The tapetal membrane in the ovule would be, for example, homologous to the vacuolate sudanophilic mass of the *Salvinia* microsporangia and to the outer membrane of the spores of *A. adiantum-nigrum*. While there may be certain, admittedly somewhat tenuous, structural similarities between the tapetal membrane of the ovules and the perispore of *Salvinia*, there are less between the former and the perispore of *A. adiantum-nigrum*.

McClymont & Larson's (1964) study of moss spores shows that the exine in these plants is less well differentiated than it is in the vascular cryptogams. At the present state of our knowledge it is probably premature to discuss whether this means that they are also more primitive in an evolutionary sense. Such a discussion would have relatively little value until we are more sure of the criteria for judging the " simple " level of organization (as opposed to " reduced " and " derived ") in spore and pollen walls. If complex structural differentiation in the exine is a measure of phylogenetic advancement then the spores of the mosses, of *Psilotum*, *Lycopodium* and the microspores of *Selaginella* have a primitive exine structure ; certainly more primitive than the pteridosperm prepollen *Schopfipollenites*, for example.

Accepting Afzelius's hypothesis (1956) that pollen grains with a thin lamellated layer in the exine are phylogenetically more advanced than those with a thick lamellated layer, it is not surprising to find that the prepollen *Schopfipollenites* is phylogenetically less advanced than the pollen of *Encephalartos*. This hypothesis would, however, be difficult to apply in cases where an inner lamellated layer is not formed. In these spores the absence of such a layer could be taken to mean either that it has been completely reduced, and therefore the spores were advanced, or that it had not developed at all. It is not clear precisely how the microspores of *Isoetes*, with a well-developed lamellated layer as the outer wall stratum, would fit into this scheme, unless one accepts Nilsson & Praglowski's (1963) suggestion that this layer is the perine (and therefore not strictly part of the exine).

From a palaeobotanical standpoint one of the most interesting facts to emerge from the present study is that of the structural similarity between the megaspores of some of the free-sporing heterosporous plants and the megaspore membranes of the fossil gymnosperms. In the heterosporous Upper Devonian pteridophyte *Archaeopteris* the exine structure of the microspore and megaspore is almost identical. The situation is made the more striking by the structural dissimilarity of the microspores and megaspores in *S. pulcherrima*. It would be convenient, to say the least, to consider the spores of *Archaeopteris* as indicating that heterospory in this plant was not far developed from a homosporous stage and that the structure of the microspores and megaspores had not differentiated much beyond that of some ancestral isospores. This situation can be contrasted with the structure of the microspores and megaspores in *Selaginella*, a plant in which heterospory has long prevailed.

It would seem that there is some correlation between a spongy or fibrous exine structure and the retention of the female gametophyte within the megaspore. This type of wall texture is seen in the megaspores of *Selaginella*, *Isoetes*, in the Carboniferous spores *Laevigatisporites* and *Cystosporites*, and also in some Palaeozoic gymno-

sperm ovules. *Didymosporites* would appear to be an exception. At the risk of overstressing the significance of this it could be surmised that the continued evolution of a megaspore of the *Archaeopteris* type could result in further development of the spongy layer and the suppression of the inner lamellated layer. This process would result from, or accompany, the retention of the female gametophyte within the megaspore. The final structure would resemble the megaspore of *Selaginella*. Resulting from the retention of the megaspore within the megasporangium to form an ovule all that would be necessary to form a megaspore membrane of the type contained in the Carboniferous pteridosperms and cordaites is the complete suppression of the thin, inner, lamellated layer and a slight reduction in thickness of the spongy layer. It is worth repeating here that this structure is totally different from that found in modern gymnospermous ovules (Pettitt 1966). The hypothesis is, however, substantially weakened by the fact that a spongy exine is not confined to the spores of free-sporing heterosporous plants and the megaspores of fossil ovules. Larson (1964) has found that the ektexine of *Linum* pollen is composed of ramifying sporopollenin rodlets and this type of structure also occurs in the pollen of *Juglans* and *Carya* (Stone *et al.* 1964).

The structural similarity of the *Archaeopteris* exine and that of the Carboniferous prepollen *Schopfipollenites* suggests that very little, if any, fine structural alteration need accompany the change from microspore to pollen grain, if indeed such a change occurred. The comparison of the prepollen exine with that of the pollen from the living cycads and conifers shows quite clearly that the exine of the fossil is ultrastructurally considerably closer to a spore than to a pollen grain.

A spore-like exine structure can also be seen in the Carboniferous pollen *Florinites* (Pl. 19, fig. 4). This material was recovered as pollen masses from the same horizon in Kansas as the cordaitalean ovules. The genus *Florinites* represents the pollen of the cordaites and has been found in unquestionably cordaitalean pollen sacs by Florin (1936), Delevoryas (1953) and Fry (1956).

Sections through the central body of the grain show that it is composed of an outer spongy layer and an inner, much thinner, granular layer (Pl. 19, fig. 5). The inner layer shows no trace of lamellation.

The structural and/or physiological significance of an inner lamellated layer in the exine is some way from being understood, but Larson's (1964) discovery that the inner wall layer of *Bruchia brevifolia* spores is lamellated only in the harmomegathic region is of significance in this connexion.

It would be of considerable interest to know the implications of fine structural changes in the exine, and to establish precisely what is responsible for the observed differences. Judging from the very apparent dissimilarity of the microspores and megaspores of *S. pulcherrima*, for example, it might be supposed that the mechanism involved is very fundamental. It is noteworthy that Manton (1950) has shown that in forms of "*Cystopteris fragilis*" a difference in spore size can be correlated with a difference in chromosome number; large spiny spores are associated with a high chromosome number ($n = 126$) and somewhat smaller spiny spores with a low chromosome number ($n = 84$). Examination with an electron microscope

would show whether the difference here is purely one of spore size or if it extends to the wall structure.

A final comment of taxonomic interest arising from this study of spore and pollen wall structure must be included.

The separation of the inner exine layer or layers that was observed in the spores of *Archaeopteris*, *Selaginella* and *Isoetes* is clearly relevant to the correct interpretation of spore and pollen grain morphology, particularly in dispersed fossil specimens. Spores in which the inner layer of the exine is separated from the outer by a cavity are described as cavate (Dettman 1963, Faegri & Iverson 1964) and at least in some cases the inner layer or membrane is termed the mesospore (see Kremp 1965 : 89). Fitting's (1900) application of this term has already been fully discussed. The amount of variation that can occur in the formation of a mesospore, even in the spores of a single sporangium, is illustrated by the microspores of *Isoetes* (Pl. 7, figs. 1–8) and *Botrychium jenmanii* Underw. (Pl. 6, fig. 6), and there is certainly a need for caution in assessing the taxonomic value of such structural features.

V. REFERENCES

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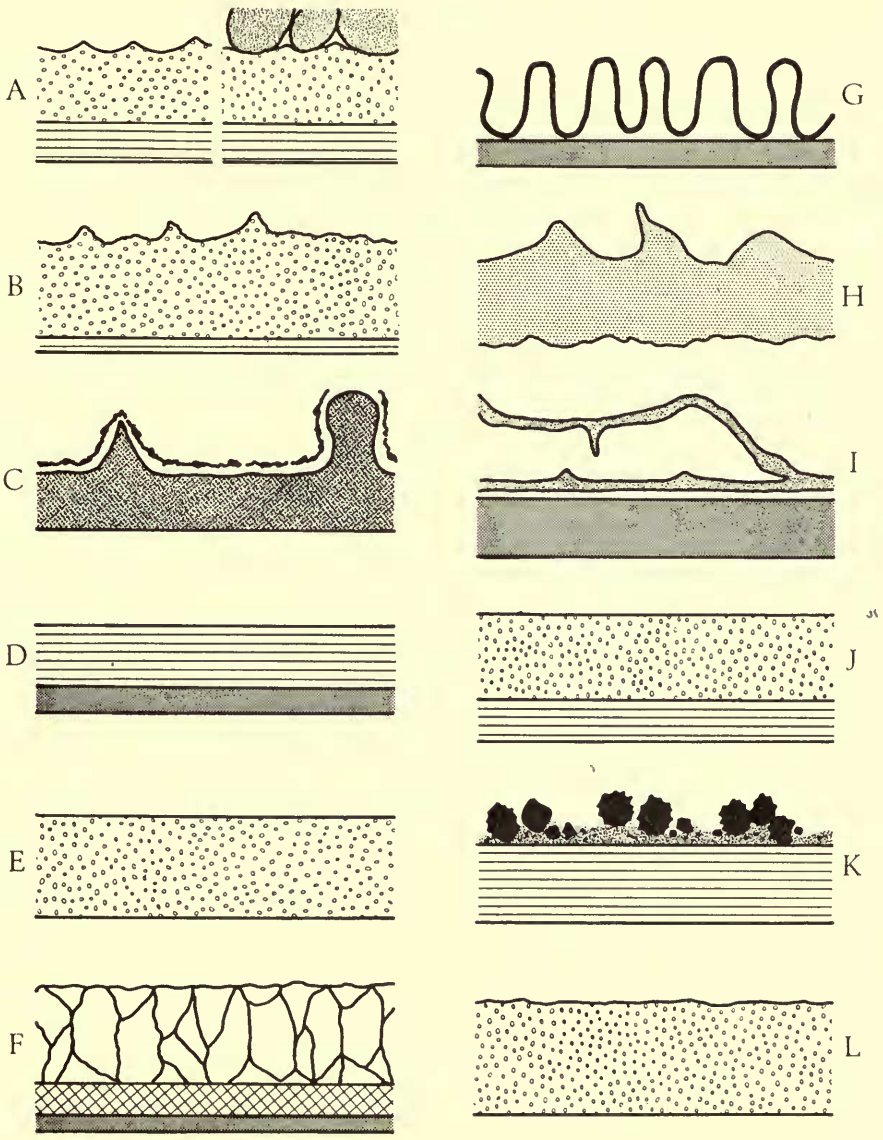

FIG. 1. Comparative diagrammatic drawings of exine stratification in : A, *Archaeopteris* microspores (left) and megaspores (right) ; B, *Selaginella* megaspores ; C, *Selaginella* microspores ; D, *Isoetes* microspores ; E, *Laevigatisporites* ; H, *Lycopodium* ; I, *Asplenium*; J, *Schopfipollenites* ; K, *Taxus* and L, a cordaite seed megaspore. The drawings are not to scale.

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PLATE 1

Selaginella pulcherrima (Megaspores) p. 227

FIG. 1. Section treated by periodic acid/Schiff procedure. Intine and thin membrane surrounding the spore protoplast coloured red. Spore protoplast is faintly pink. The exine is not coloured. ×400.

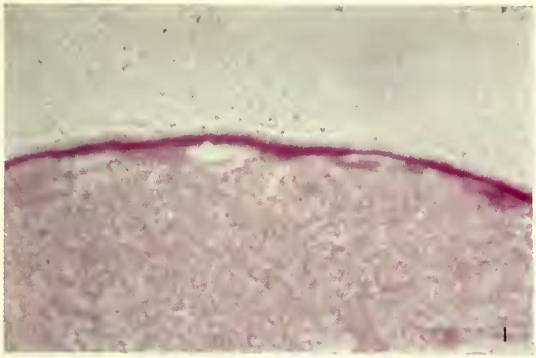
FIG. 2. Section treated by the periodic acid/Schiff procedure. The thin membrane (red) surrounding the spore protoplast is shown more clearly than in Fig. 1. ×425.

FIG. 3. Section treated by periodic acid/Schiff procedure. The inner, fibrous zone of the mesospore is coloured a vivid red. The outer, granular zone, is not coloured. ×460.

FIG. 4. Section treated by Nile blue sulphate method. Ektexine and outer, granular zone of mesospore stained blue. Inner, fibrous zone of mesospore very faintly coloured. ×157.

FIG. 5. Section stained with Sudan black B. The mesospore and the ektexine are strongly sudanophilic. ×390.

FIG. 6. Section stained with acetylated Sudan black B. Three sudanophilic membranes can be seen ; the ektexine (black arc at top of figure), the endexine and the one closely associated with the spore protoplast. The intine is not stained. Protoplast and inner wall layers have pulled away from the ektexine. ×685.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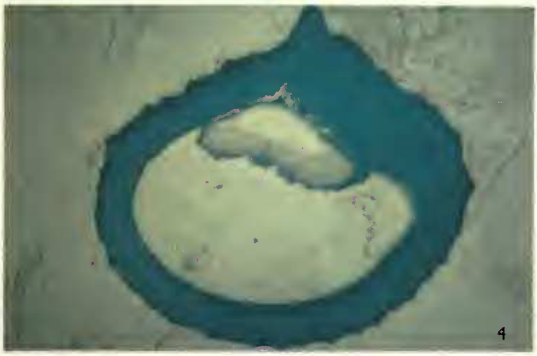
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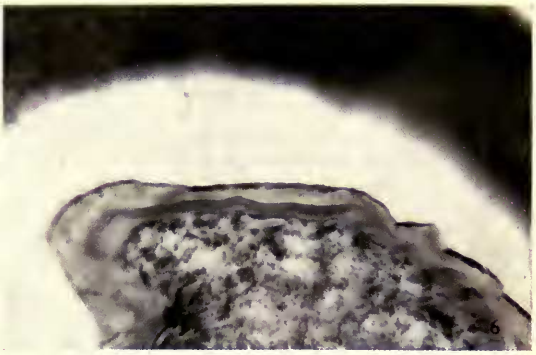
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PLATE 2

Archaeopteris cf. ***jacksoni*** Dawson p. 225

FIG. 1. Outer part of megaspore wall showing associated spherical bodies. ×10,000.
FIG. 2. Inner part of megaspore wall. ×30,000.

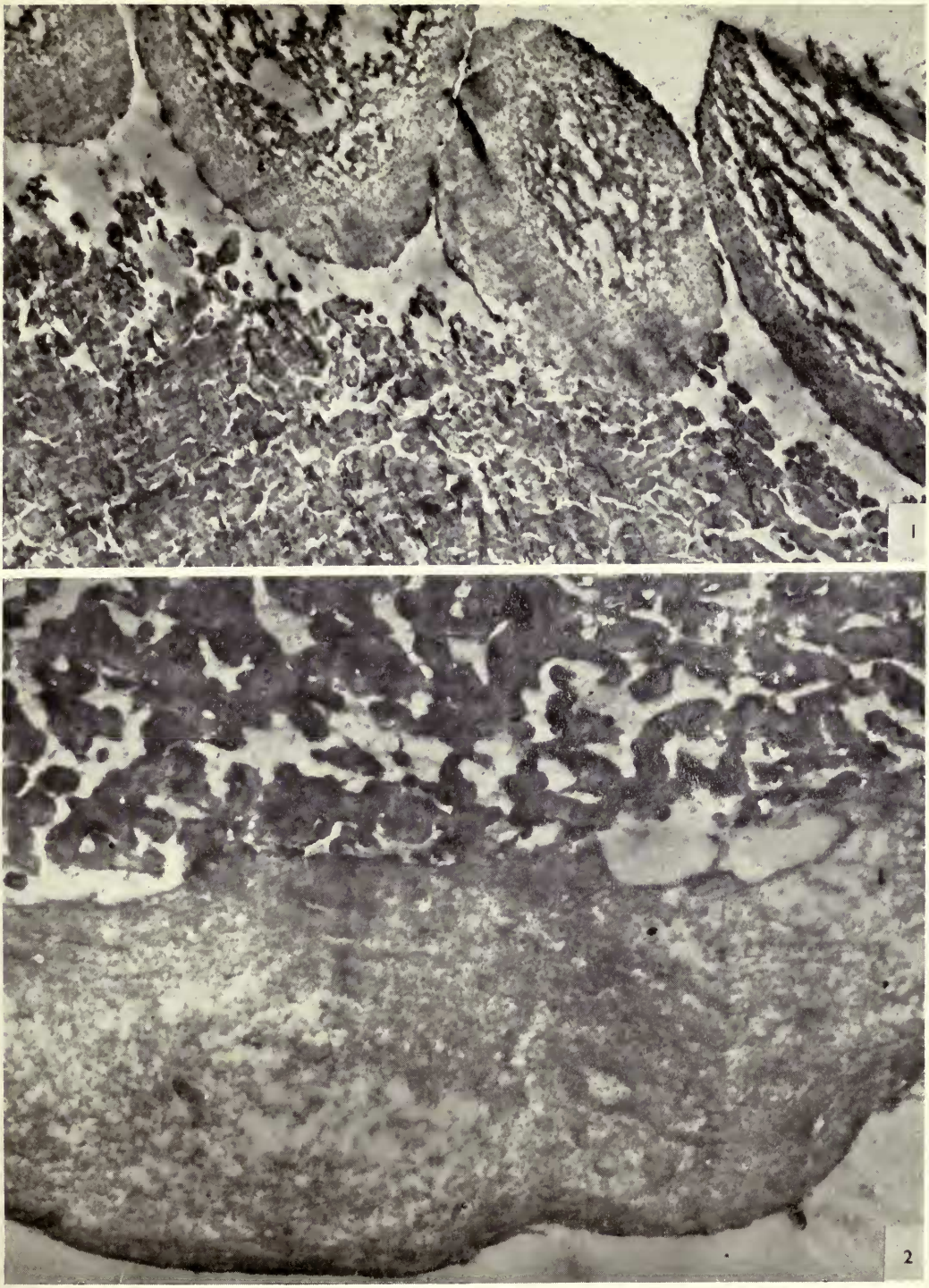
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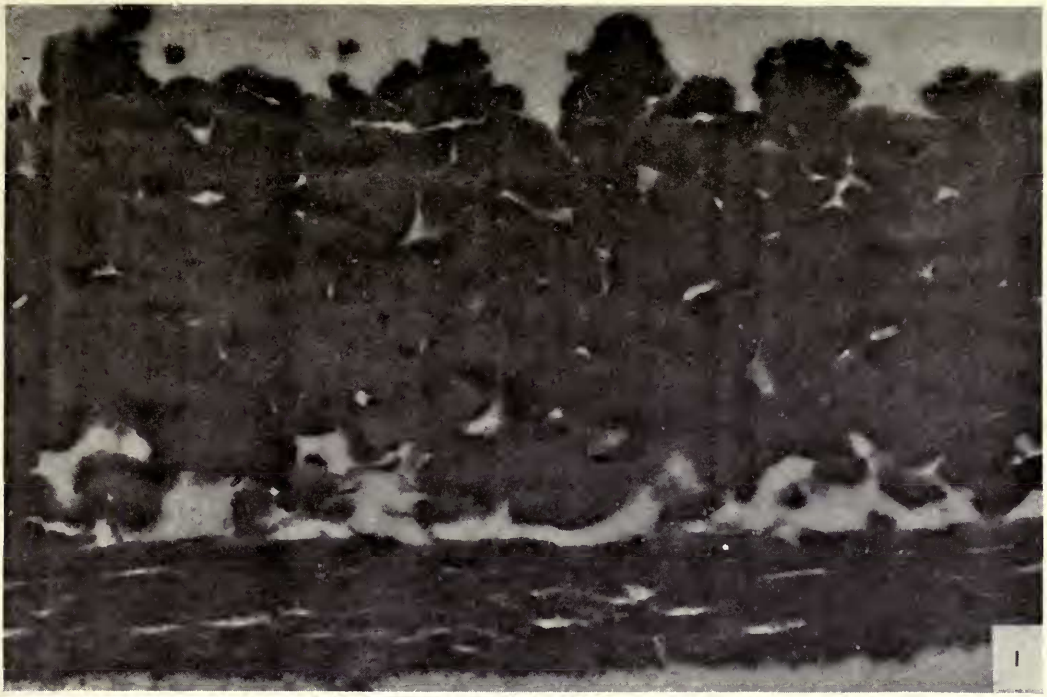
PLATE 3

Archaeopteris cf. ***jacksoni*** Dawson p. 225

FIG. 1. Section of microspore wall. ×30,000.

FIG. 2. Section of microspore wall passing through a commissure. The endexine passes upwards through the opening and overlaps onto the outer surface of the spore. ×7,500.

FIG. 3. Section of megaspore wall showing separation of the endexine into two layers. ×15,000.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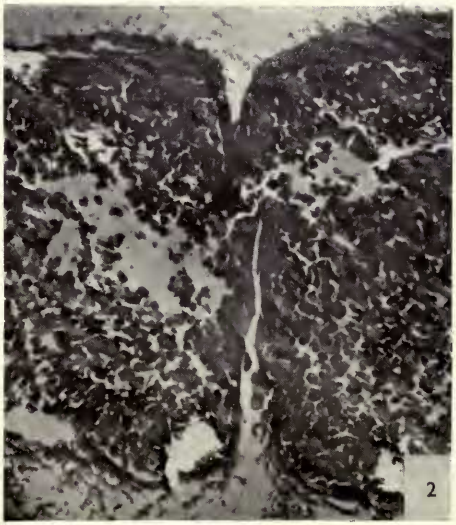
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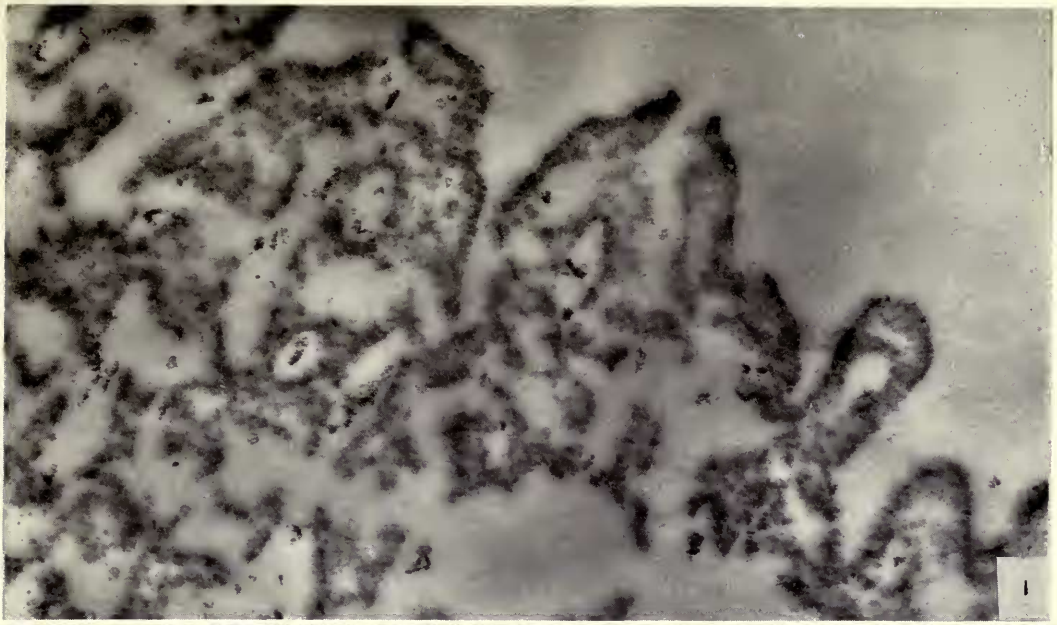
PLATE 4

Selaginella pulcherrima Liebm. p. 227

FIG. 1. Outer part of megaspore wall. ×25,000.

FIG. 2. Inner part of megaspore wall. The spore lumen is to the lower right of the micrograph. ×11,250.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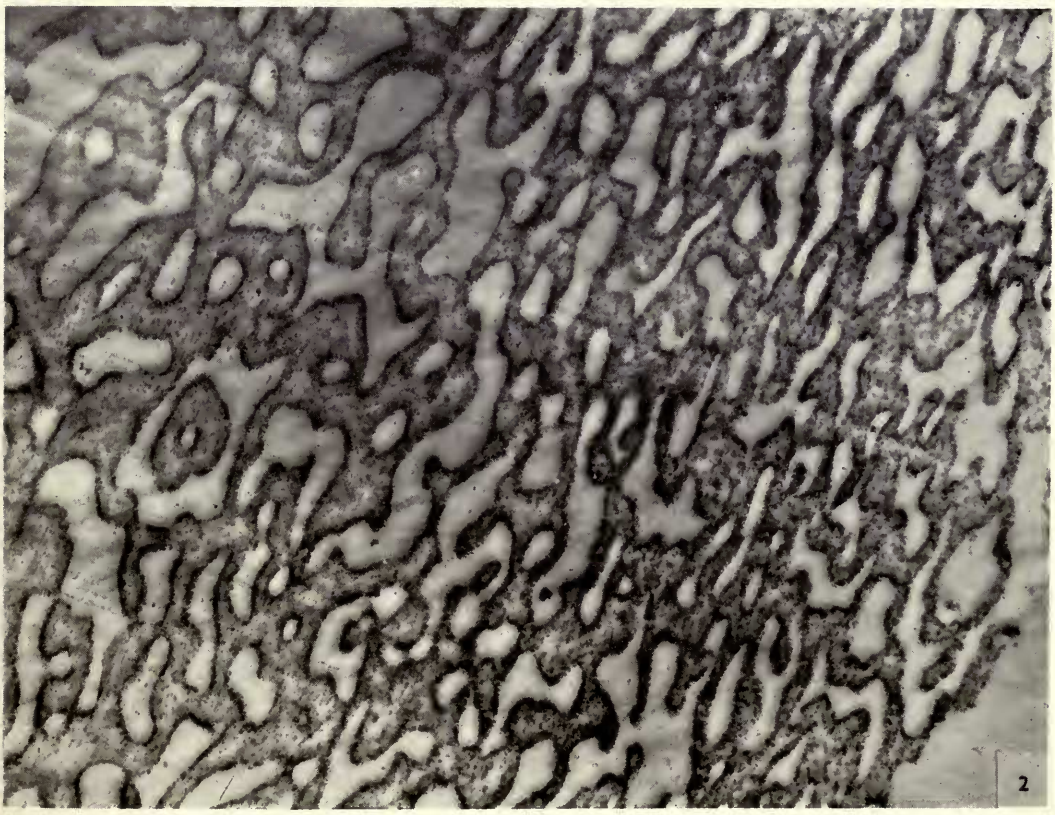
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PLATE 5

Selaginella pulcherrima Liebm. p. 227

FIG. 1. Section of an acetolyzed megaspore wall showing structure of ektexine. The endexine is present as a thin, phase-white layer. Anoptral contrast. × 1,000.

FIGS. 2, 3. Sections of megaspores treated with mercuric bromophenol blue. The spore protoplast in Fig. 2 and the mesospore in Fig. 3 are coloured blue-green. Fig. 2, × 100. Fig. 3, × 128.

FIG. 4. Acetolyzed megaspore containing a mesospore. × 100.

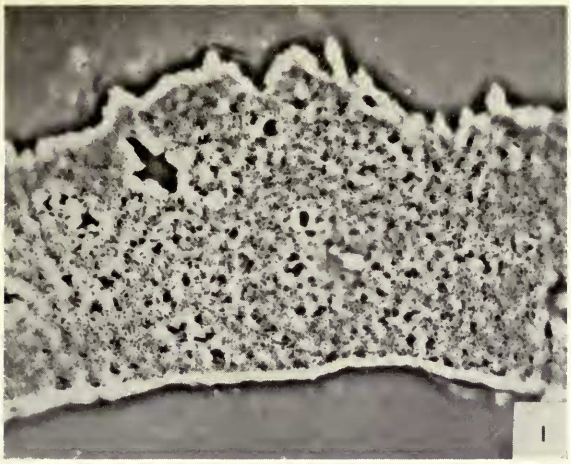
FIG. 5. Tangential section through proximal region of megaspore. The endexine lines the triradiate commissures. × 125. Stained with Bismark brown.

FIG. 6. Section of microspore wall. × 7,500.

Isoetes humilior F. Müll. p. 232

FIG. 7. Section of acetolyzed megaspore wall. Anoptral contrast, × 1,000.

FIG. 8. Section of acetolyzed megaspore wall showing separation of inner part to form a distinct membrane. See also Pl. 6, fig. 1. Anoptral contrast, × 1,000.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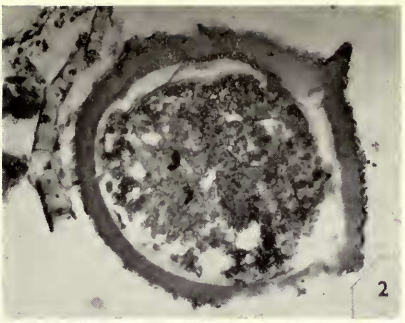
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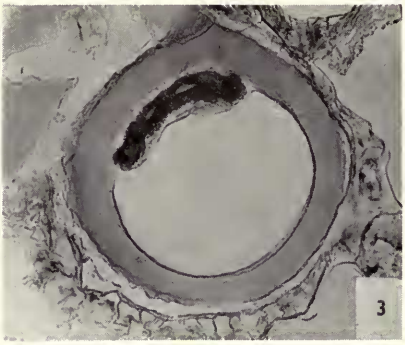
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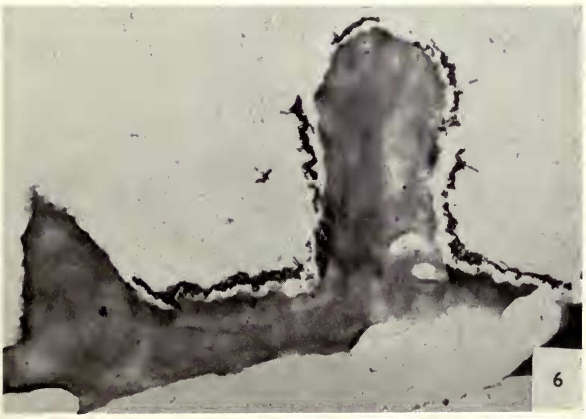
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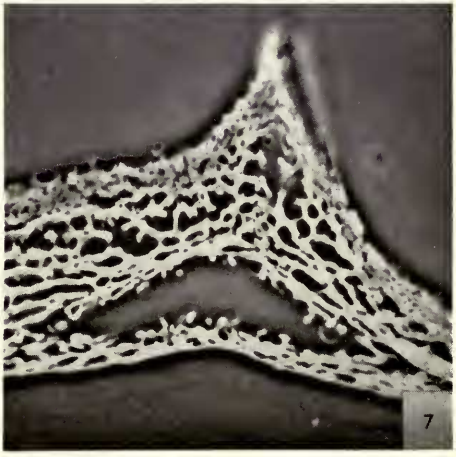
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PLATE 6

Isoetes humilior F. Müll. p. 232

FIG. 1. Section of acetolyzed megaspore showing partial separation of inner part of wall. Anoptral contrast, ×106.

Isoetes echinospora Dur. p. 234

FIG. 2. Section of acetolyzed microspore wall. ×10,000. Section stained with potassium permanganate.

FIG. 3. Section of acetolyzed microspore wall showing separation of inner homogeneous layer from outer lamellated layer. ×7,000.

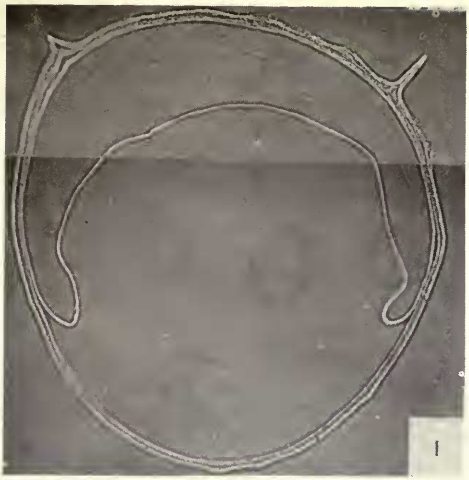
Laevigatisporites cf. ***glabratus*** (Zerndt) p. 235

FIG. 4. Entire megaspore from Lawrence Shale, Kansas. ×20.

FIG. 5. Section of wall. Phase contrast, ×640.

Botrychium jenmanii Underw. p. 254

FIG. 6. Acetolyzed spores showing separation of wall layers. See also Pl. 7, fig. 9. ×370.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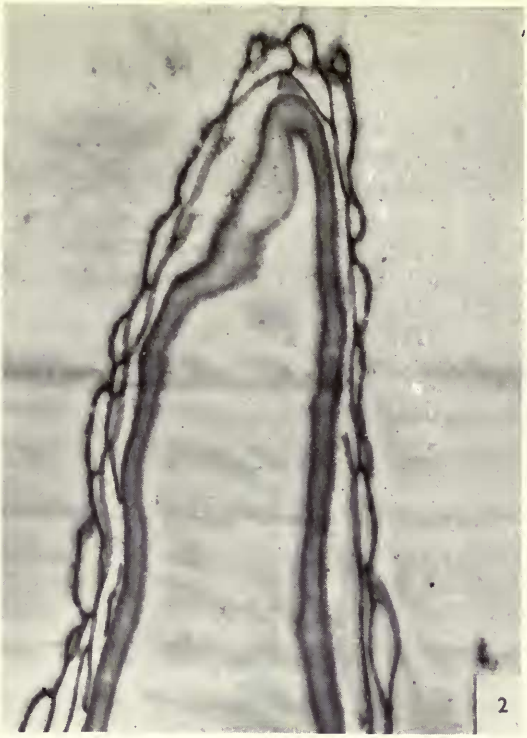
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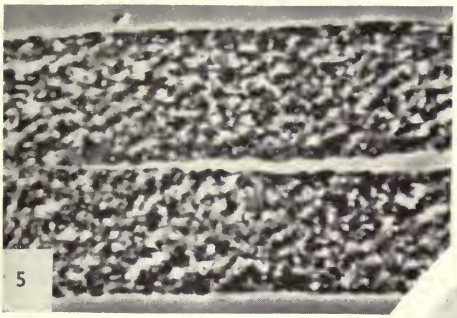
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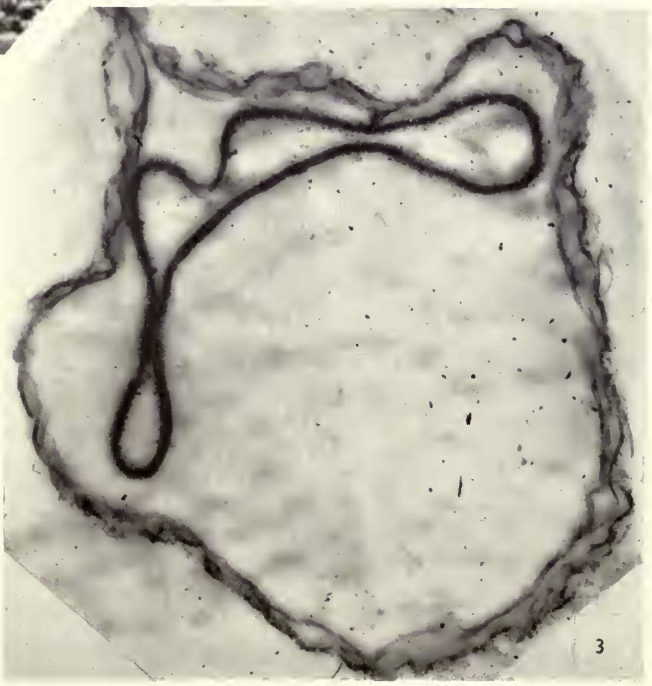
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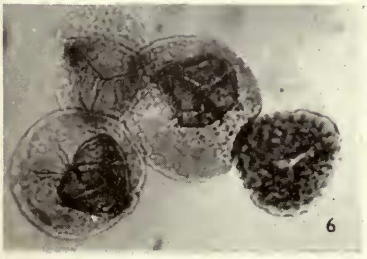
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PLATE 7

Isoetes echinospora Dur. p. 234

FIGS. 1–8. Acetolyzed microspores showing (except in Fig. 1) separation of wall layers. Figs. 1, 2, 5, 6, 8 sections under phase contrast. Figs. 3, 4, 7 entire spores. For full explanation see text. Fig. 1. ×2,200; Figs. 2, 4. ×1,700; Fig. 3, ×1,500; Fig. 5. ×2,100; Fig. 6, ×1,900; Fig. 7. ×1,000; Fig. 8. ×1,850.

Botrychium jenmanii Underw. p. 254

FIG. 9. Section of acetolyzed spore showing separation of wall layers. See also Pl. 6, fig. 6. Phase contrast, ×925.

Laevigatisporites cf. ***glabratus*** (Zerndt) p. 235

FIG. 10. Section of wall. ×30,000.

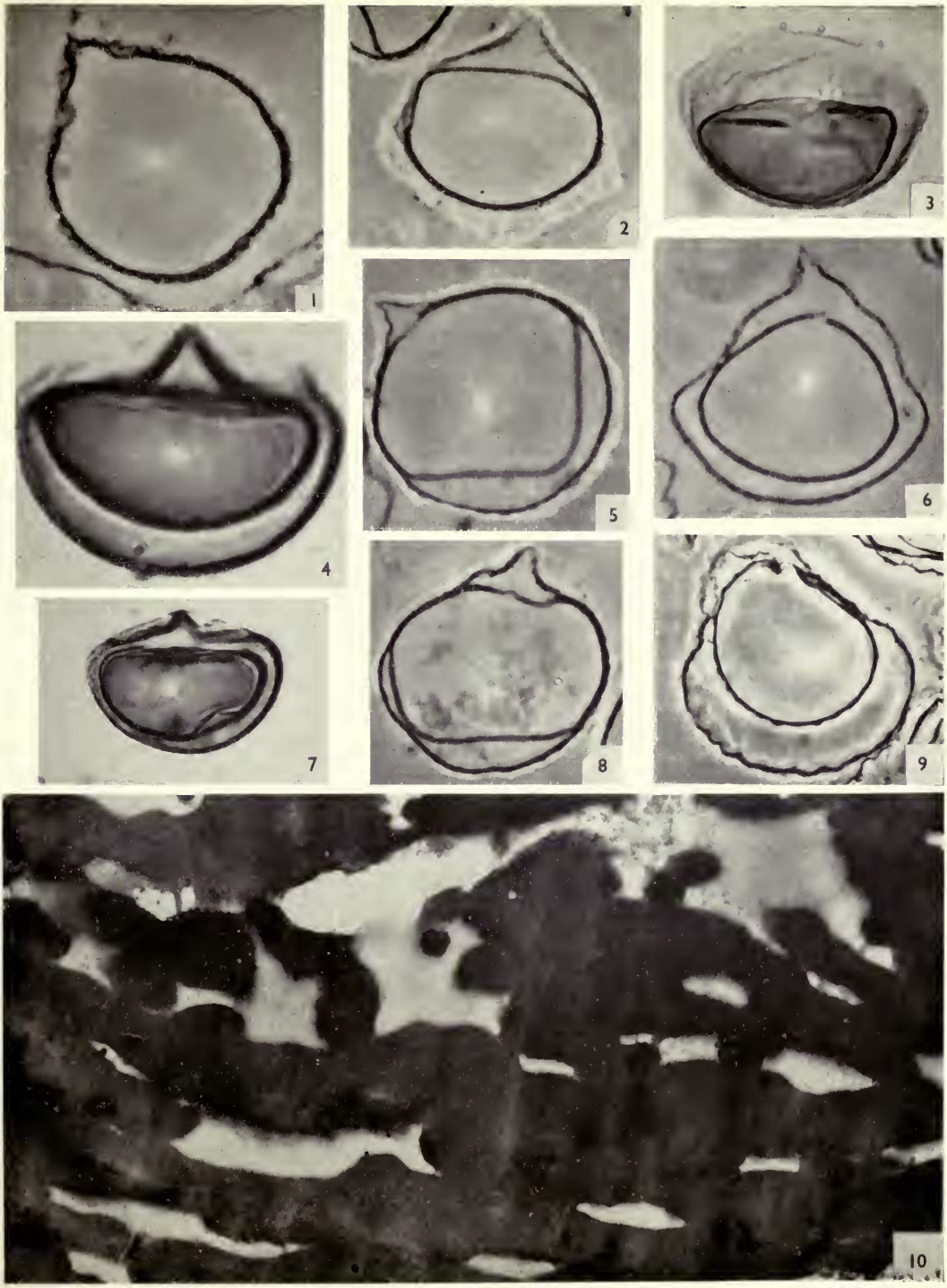
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PLATE 8

Marsilea drummondii A. Br. p. 235

FIG. 1. Entire, acetolyzed megaspore. ×42.
FIG. 4. Section of acetolyzed megaspore wall. Phase contrast, ×460.

Marsilea quadrifolia L. p. 235

FIGS. 2, 3. Sections of acetolyzed megaspore wall. Fig. 2 is the inner part and Fig. 3 the outer. Both micrographs ×10,000. Sections stained with potassium permanganate.

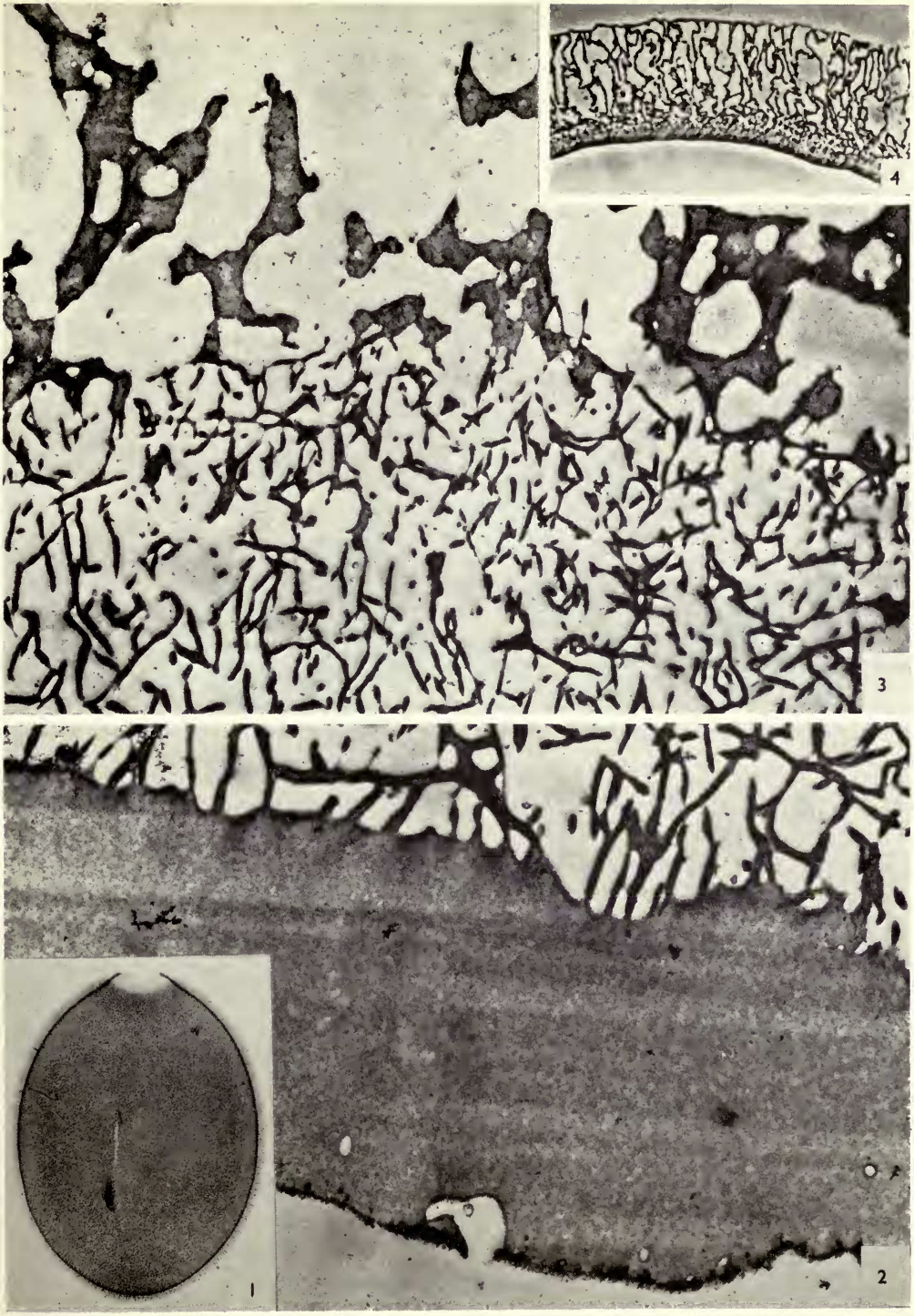
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PLATE 9

Marsilea quadrifolia L. p. 235, 237

FIG. 1. Apex of acetolyzed megaspore showing abortive spores. ×310.

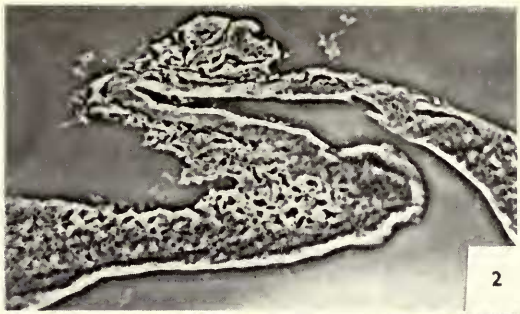
FIGS. 4, 5. Sections of acetolyzed microspore walls. Fig. 4, ×15,000. Fig. 5, ×12,500. Sections stained with potassium permanganate.

Marsilea drummondii A. Br. p. 235, 237

FIG. 2. Section through apex of acetolyzed megaspore showing structure of abortive spores. Anoptral contrast, ×470.

FIG. 3. Section of acetolyzed microspore wall. ×7,500.


1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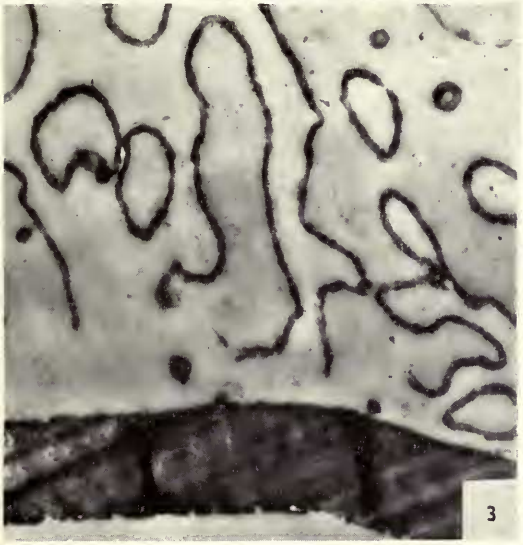
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4

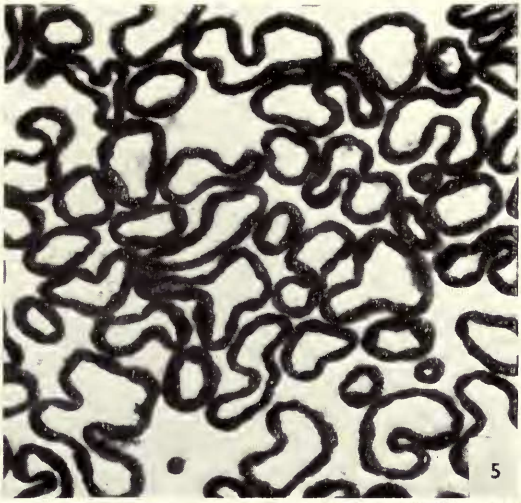

5

PLATE 10

Marsilea drummondii A. Br. p. 237

FIG. 1. Mass of normal and abortive spores from acetolyzed microsporangium. ×103.
FIG. 2. Acetolyzed microspore. ×333.

Marsilea quadrifolia L. p. 237

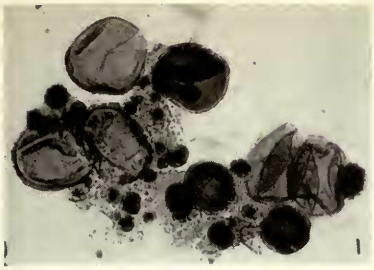
FIGS. 3–8. Sections of acetolyzed microspores showing variation in development of inner wall layer. For full explanation see text. All are anoptral contrast pictures. Fig. 3. ×520; Fig. 4. ×800; Fig. 5. ×880; Fig. 6. ×590; Fig. 7. ×500; Fig. 8. ×600.

Regnellidium diphyllum Lindm. p. 240

FIGS. 9, 10. Sections of acetolyzed megaspore wall. Phase contrast. Fig. 9. ×660; Fig. 10. ×1,300.

Lycopodium selago L. p. 241

FIG. 11. Section of acetolyzed spore wall. ×5,000.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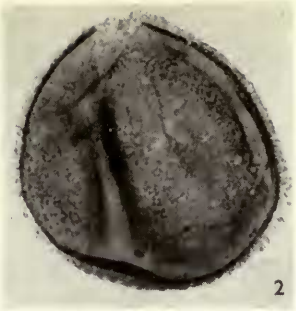
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3


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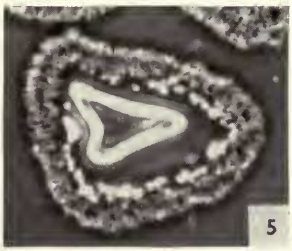
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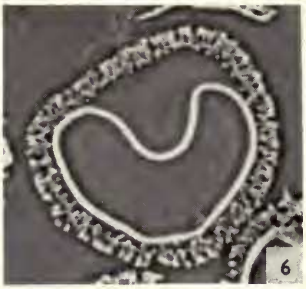
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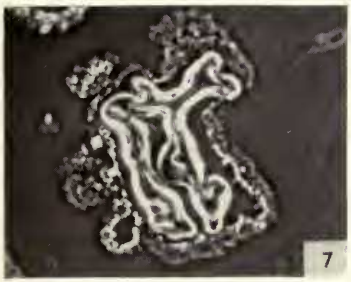
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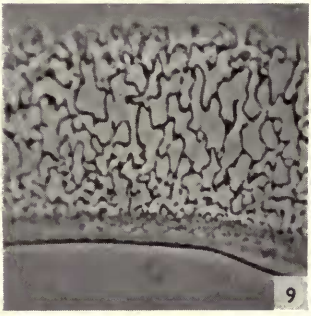
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10


11

PLATE 11

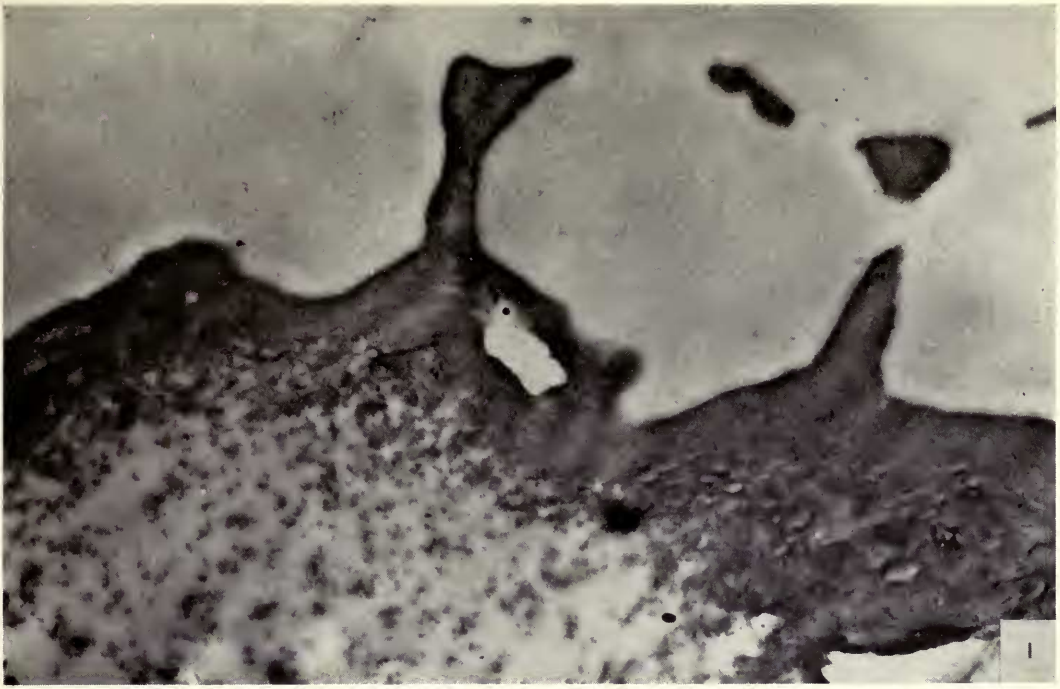
Lycopodium selago L. p. 241

FIG. 1. Section of acetolyzed spore wall. ×30,000.

Psilotum nudum Griseb. p. 242

FIG. 2. Entire, acetolyzed spore. ×440.

FIG. 3. Section of acetolyzed spore wall. ×10,000. (Section stained with lead hydroxide.)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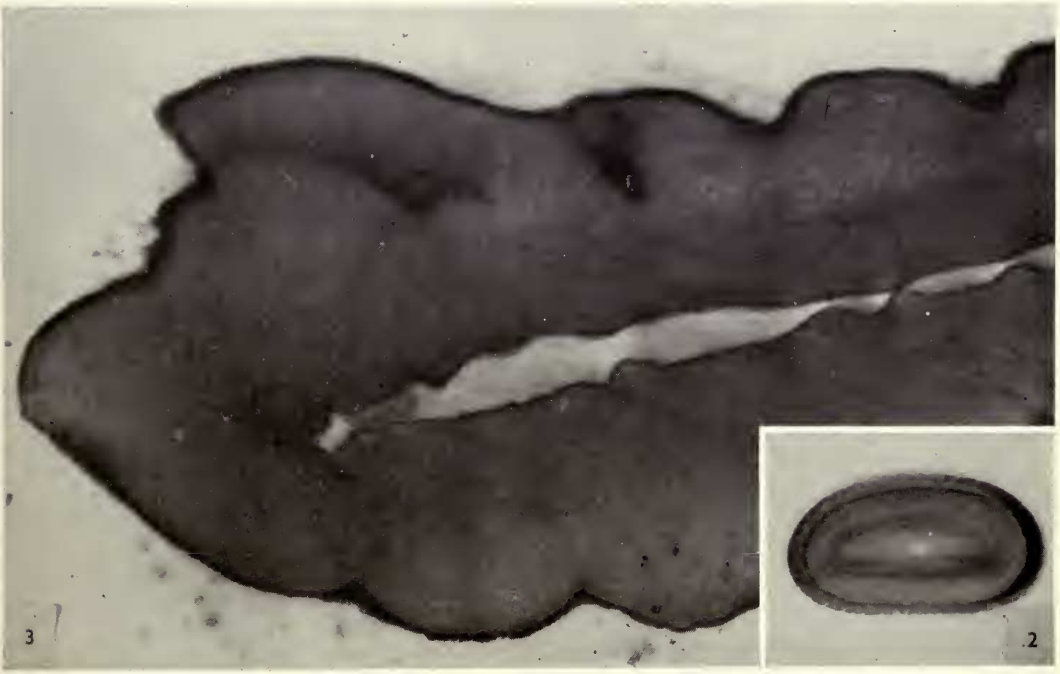
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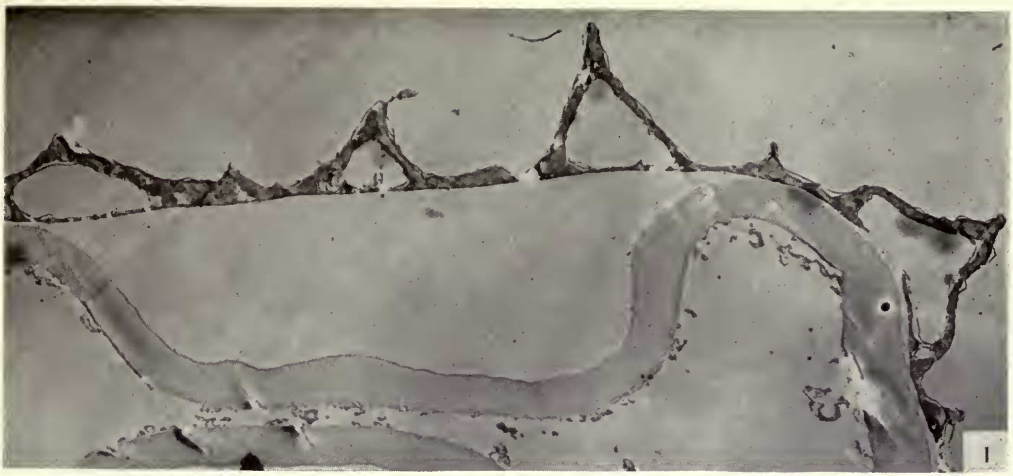
PLATE 12

Asplenium adiantum—nigrum L. p. 242

FIGS. 1, 2. Sections of acetolyzed spore walls. Fig. 1, ×3,000. Fig. 2, ×12,500.
FIG. 5. Entire, acetolyzed spore. ×770.

Archaeotriletes sp. p. 243

FIGS. 3, 4. Sections of wall. Fig. 3, ×10,000. Fig. 4, ×6,250.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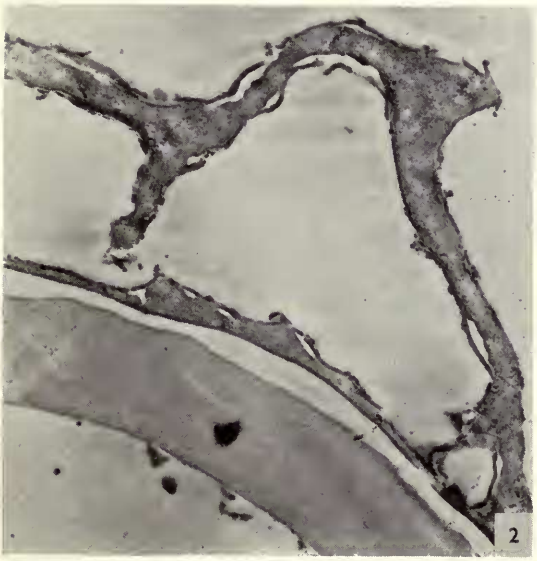
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4

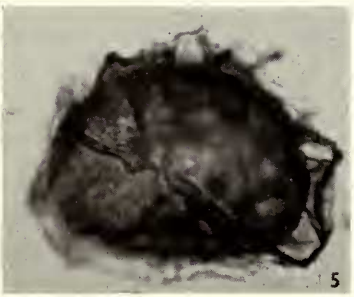
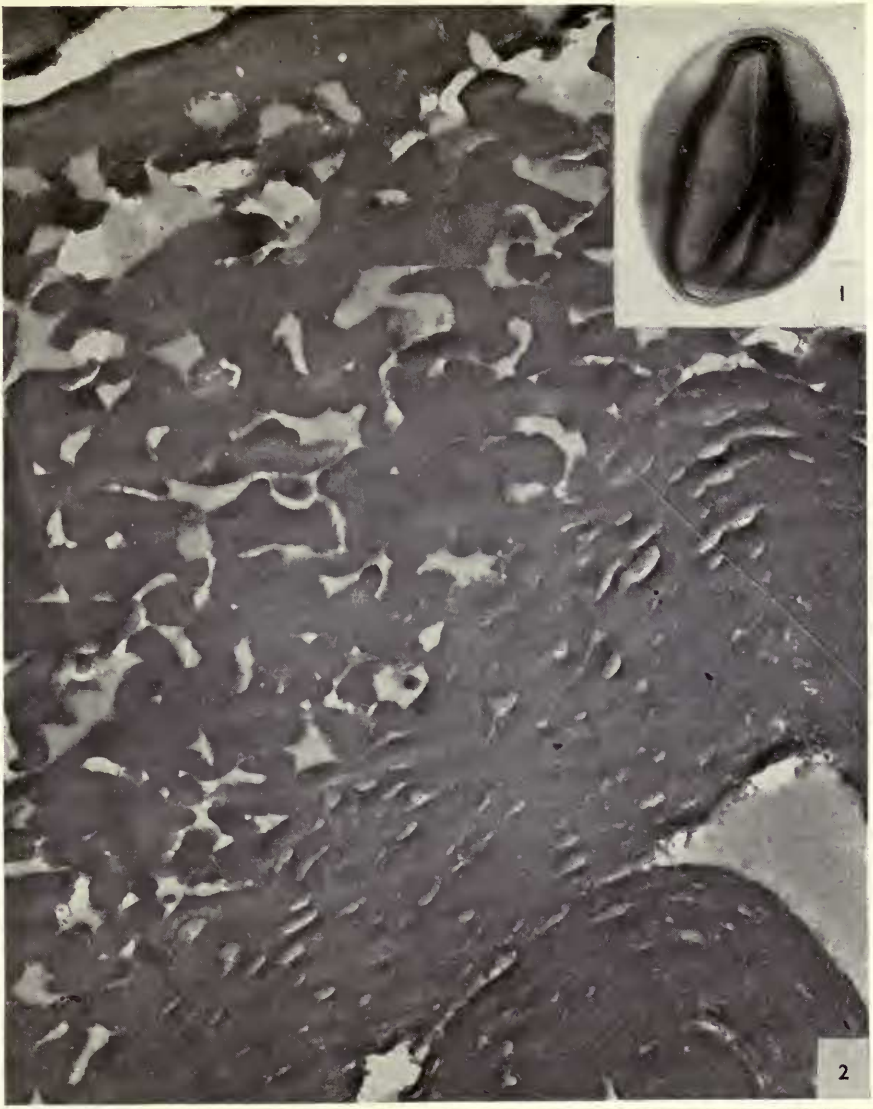

5

PLATE 13

Schopfipollenites sp. p. 243

FIG. 1. Entire grain from Lawrence Shale, Kansas. ×200.

FIGS. 2, 3. Sections of wall. The outer edge of the grain is at the top left of fig. 2, the lumen is at the bottom right. Fig. 2, ×22,500. Fig. 3, ×4,000.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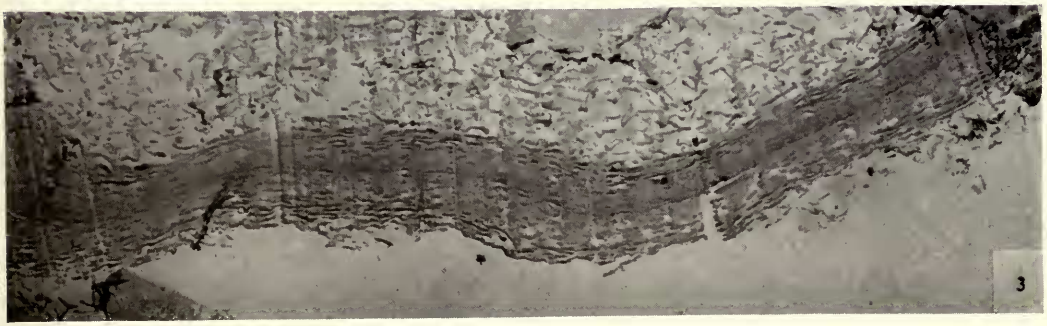

3

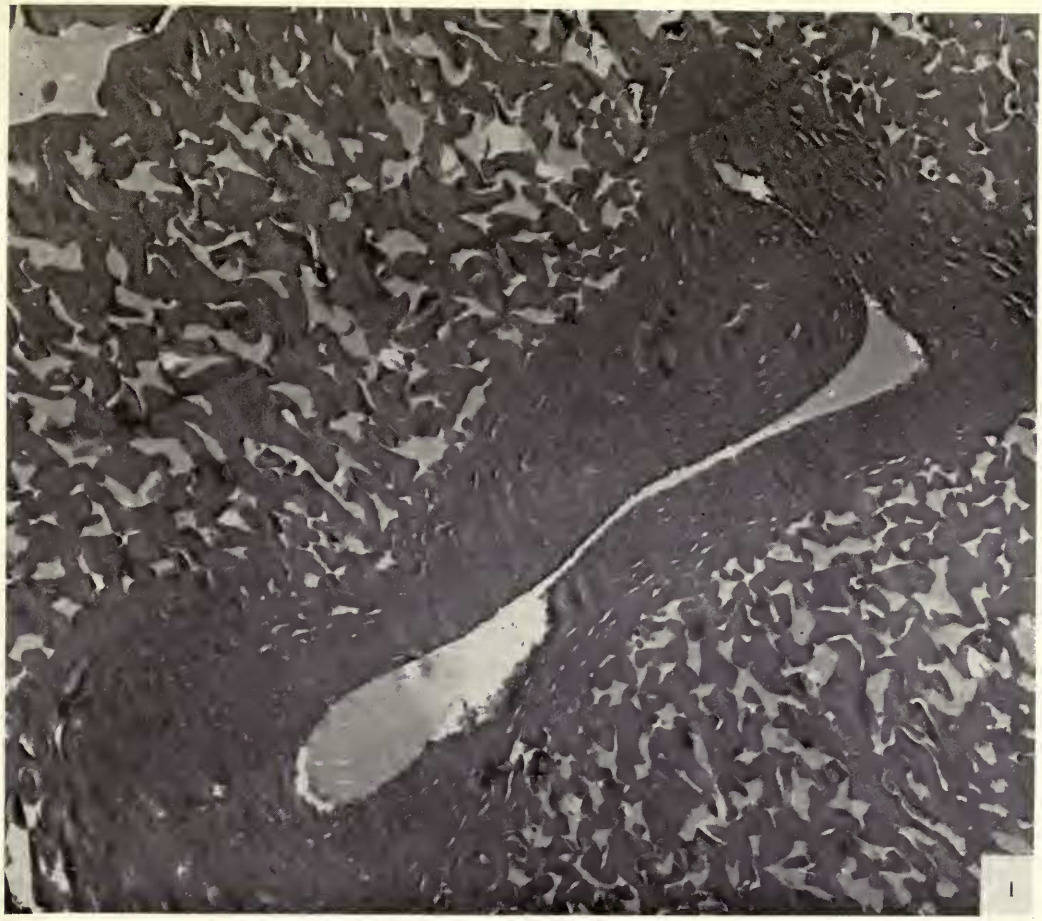
PLATE 14

Schopfipollenites sp. p. 243

FIG. 1. Sections of the wall. ×10,000.

Encephalartos villosus Lem. p. 244

FIG. 2. Section of acetolyzed pollen wall. ×10,000.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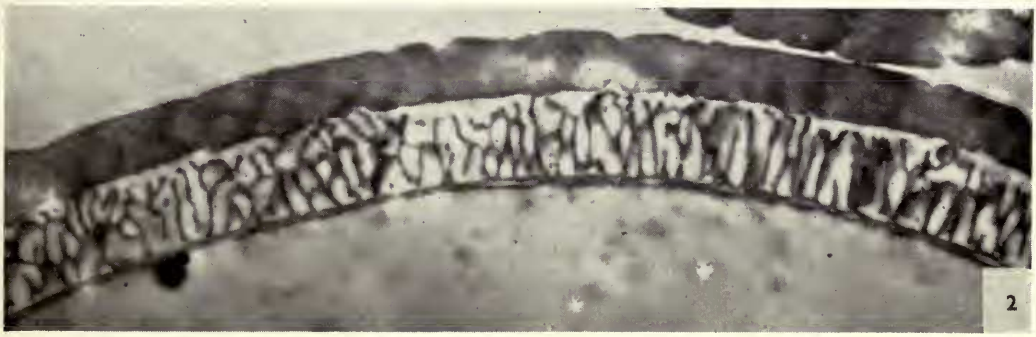
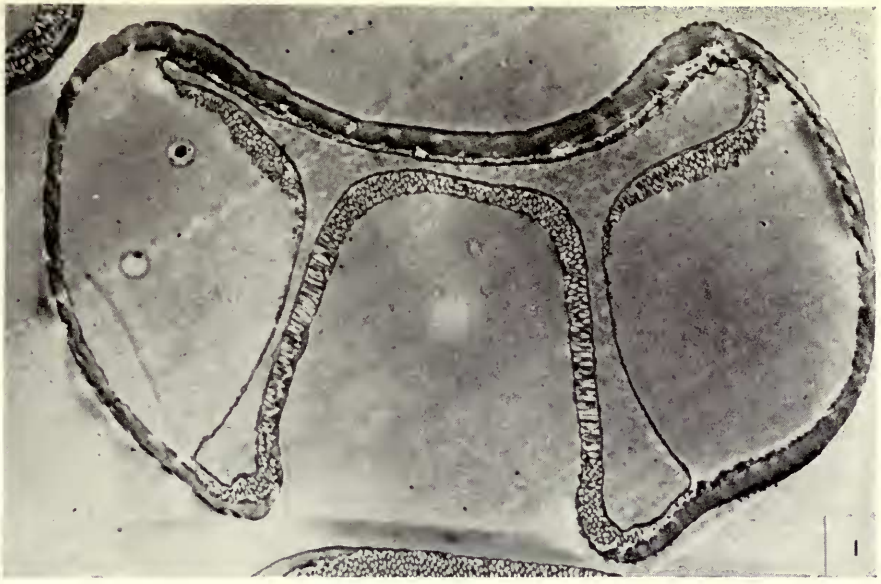

2

PLATE 15

Encephalartos villosus Lem. p. 244

FIGS. 1, 2. Sections of acetolyzed pollen walls. Fig. 1 is more or less transverse and Fig. 2 more or less longitudinal. Both micrographs × c. 2,500.

FIG. 3. Entire, acetolyzed pollen grain. × 1,600.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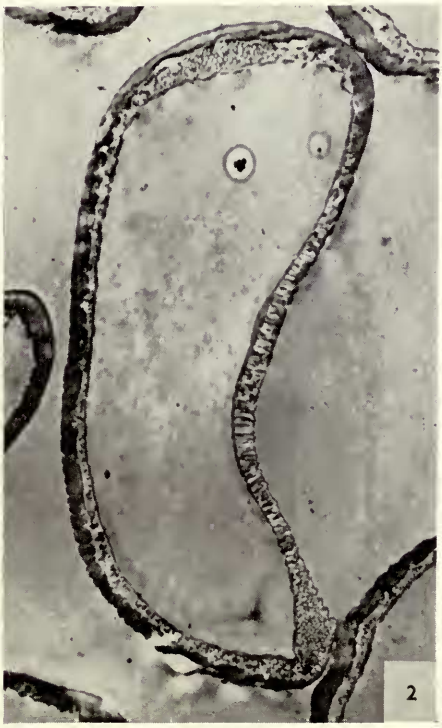
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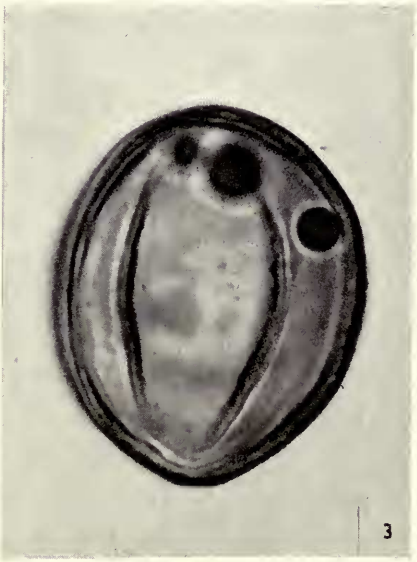
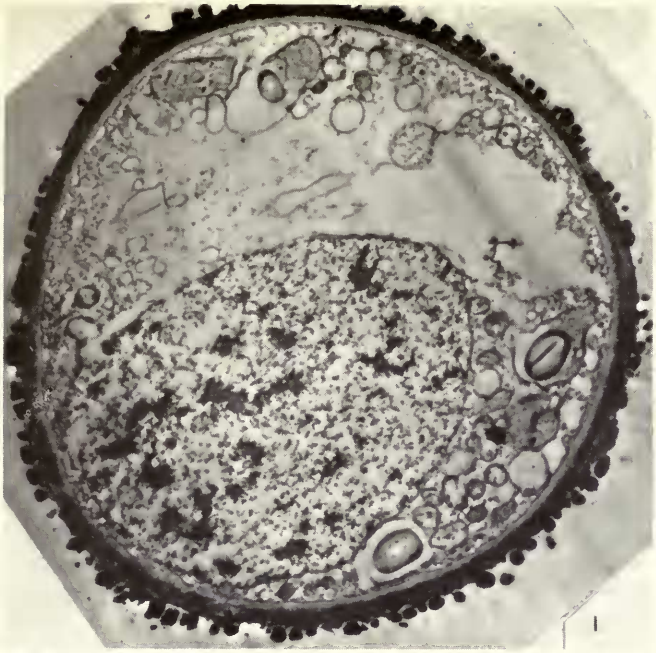

3

PLATE 16

Taxus baccata L. p. 245

FIGS. 1–3. Sections of pollen grains fixed in glutaraldehyde and post-fixed in osmium tetroxide. Fig. 1, ×c. 6,000. Fig. 2, ×20,000. Fig. 3, ×15,000.

FIG. 4. Micrograph showing small, electron-dense droplets in tapetal cell cytoplasm. (Fixed in glutaraldehyde, post-fixed in osmium tetroxide.) ×15,000.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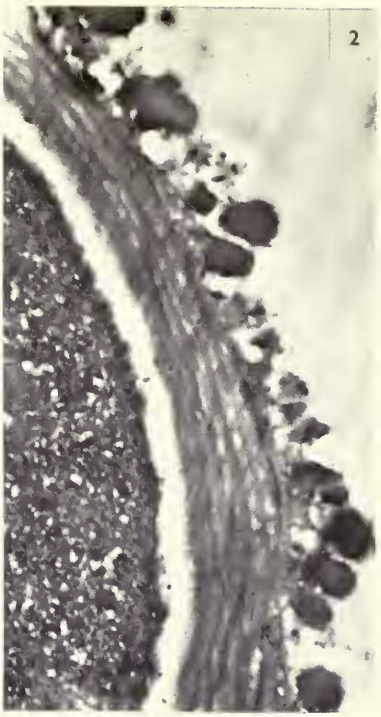
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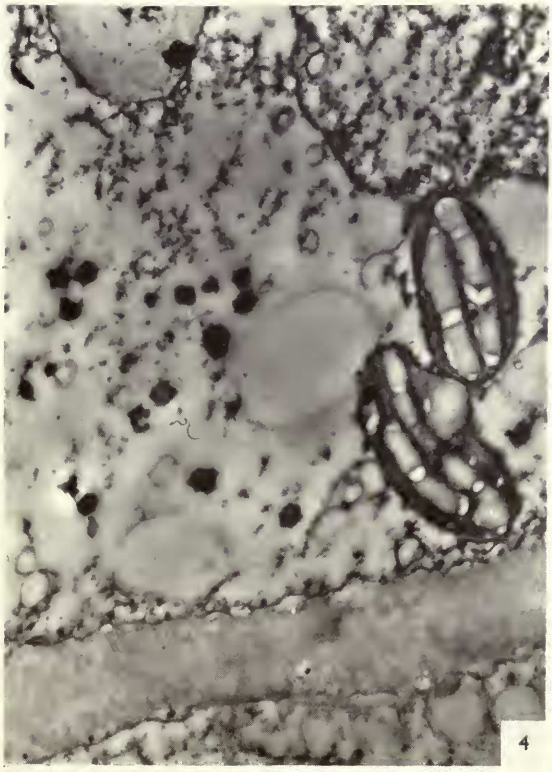
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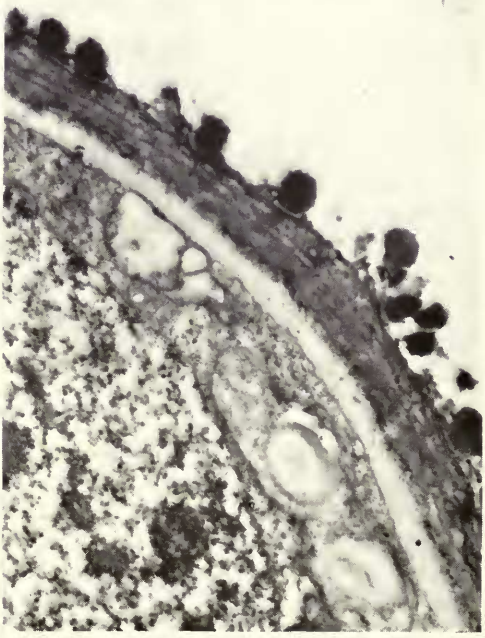
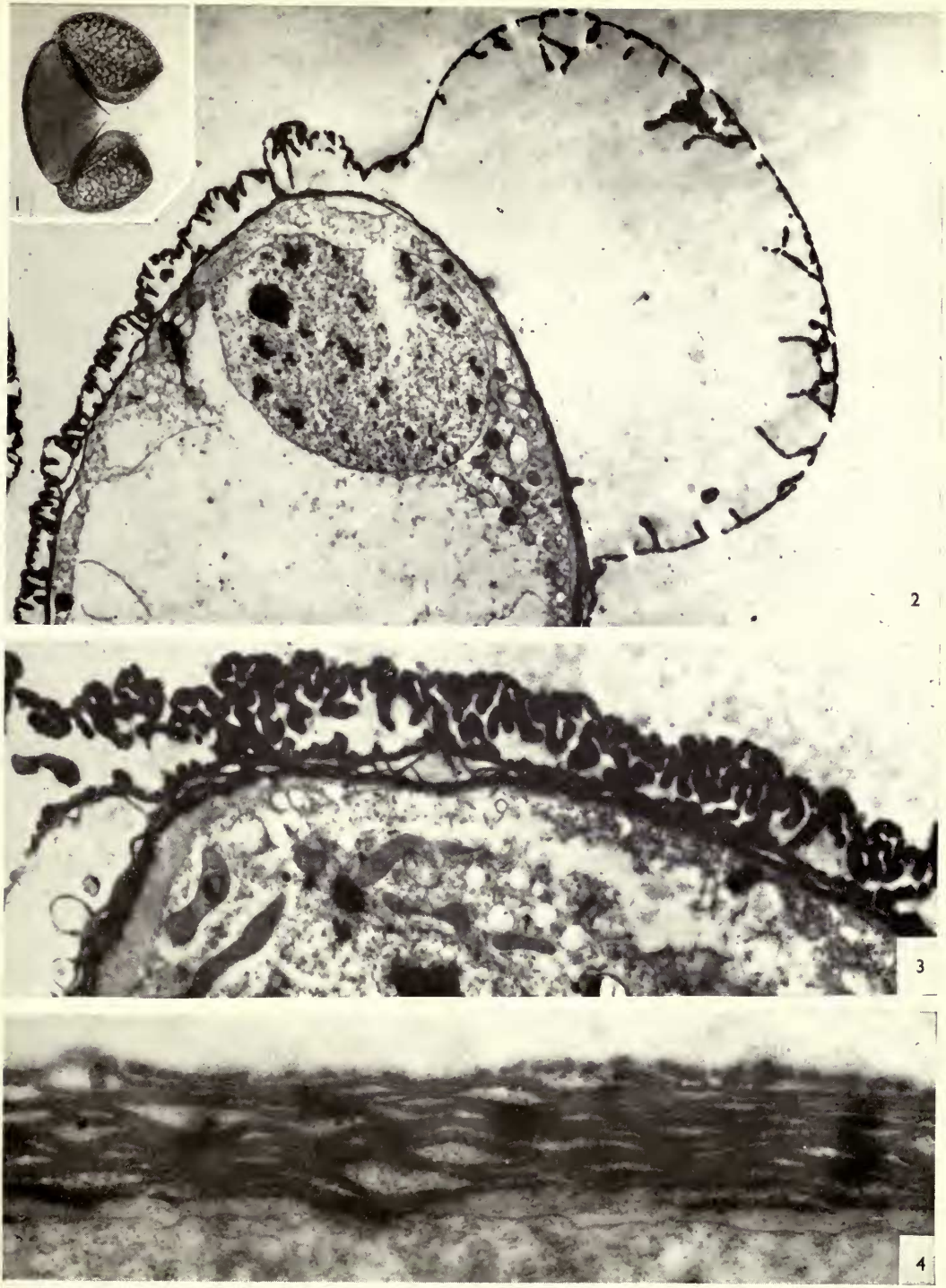

3

PLATE 17

Pinus sylvestris L. p. 246

FIG. 1. Entire, acetolyzed pollen grain. ×280.

FIGS. 2–4. Sections of pollen grains fixed in glutaraldehyde and post-fixed in osmium tetroxide. Fig. 4 shows the " foot layer " forming the floor of the saccus and the underlying endexine. Fig. 2, × c. 2,000. Fig. 3, ×7,500. Fig. 4, ×30,000.


1
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4

PLATE 18

Pinus sylvestris L. p. 246

FIG. 1. Section of pollen grain showing origin of a saccus. (Fixed in glutaraldehyde and post-fixed in osmium tetroxide.) × 10,000.

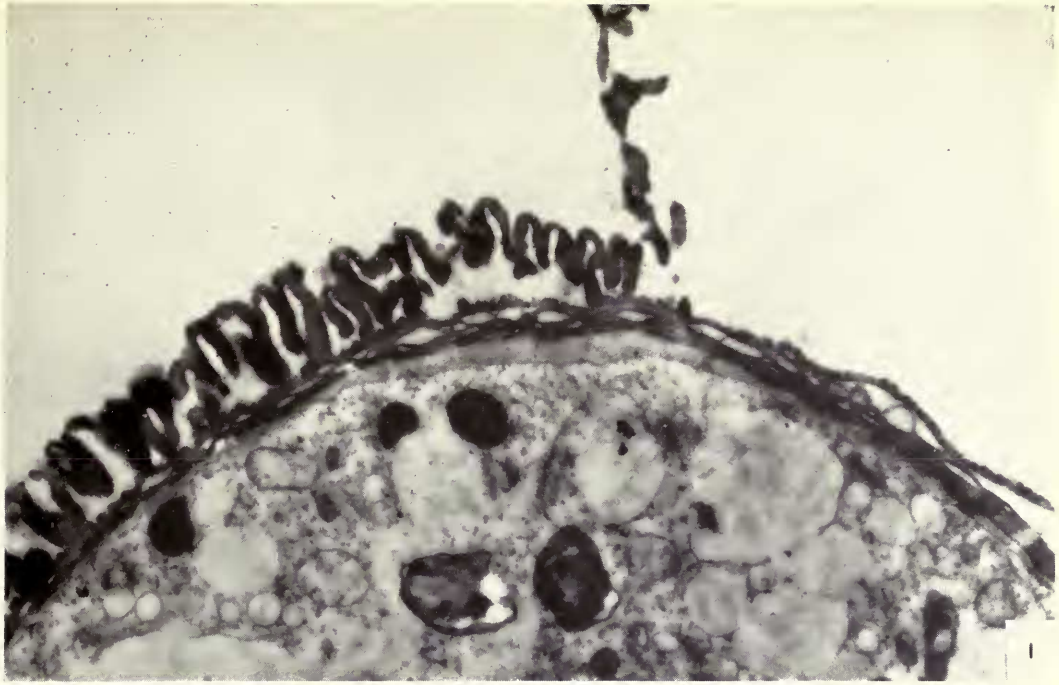
FIG. 2. Micrograph showing the Ubisch body-like objects adjacent to tapetal cell wall. Part of saccus of pollen grain forms an arc in lower part of figure. (Fixed in glutaraldehyde and post-fixed in osmium tetroxide.) × 7,500.

Trigonocarpus sp. p. 247

FIG. 3. Section of megaspore membrane. Anoptral contrast, × 570.

Taxospermum undulatum Neely p. 247

FIG. 4. Surface view of megaspore membrane. Phase contrast, × c. 600.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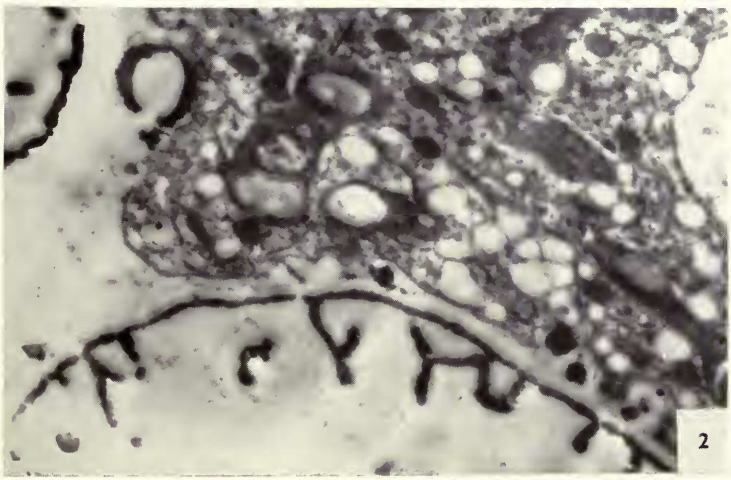
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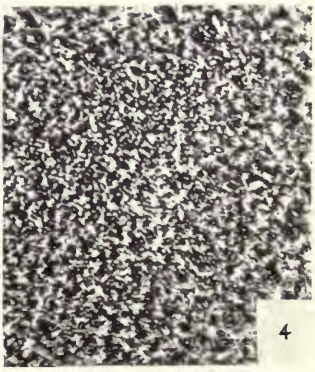

4


3

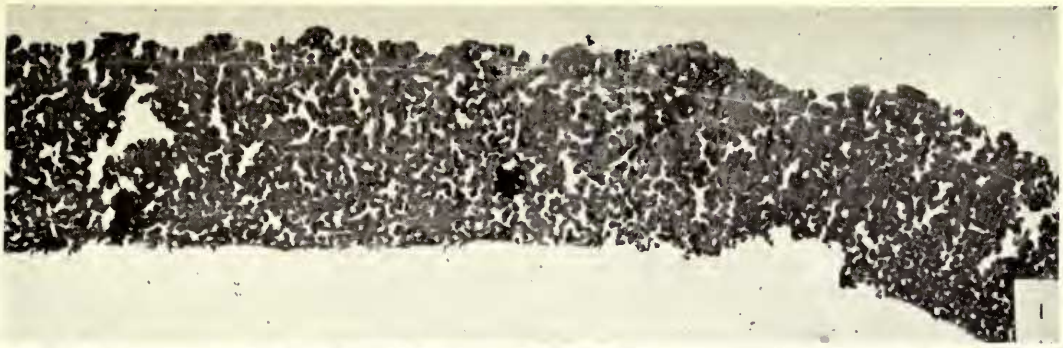
PLATE 19

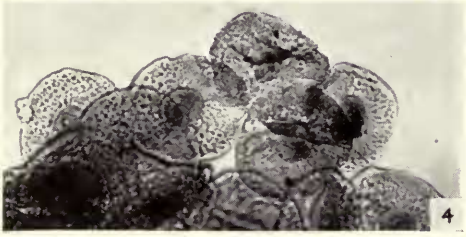
FIGS. 1–3. Sections of megaspore membrane from cordaite ovule. Fig. 3 shows the inner edge of the membrane. Fig. 1, × c. 2,000. Fig. 2, ×30,000. Fig. 3, ×6,250.

Florinites sp. p. 253

FIG. 4. A mass of pollen grains from Lawrence Shale, Kansas. ×500.

FIG. 5. Section of pollen wall. The inner, granular layer is towards the bottom of the micrograph. ×10,000.





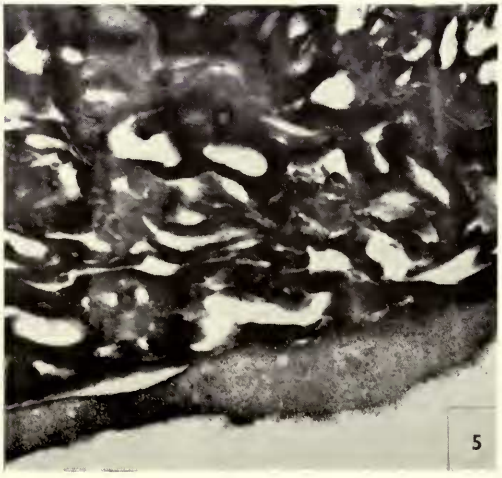


PLATE 20

Cystosporites giganteus (Zerndt) p. 248

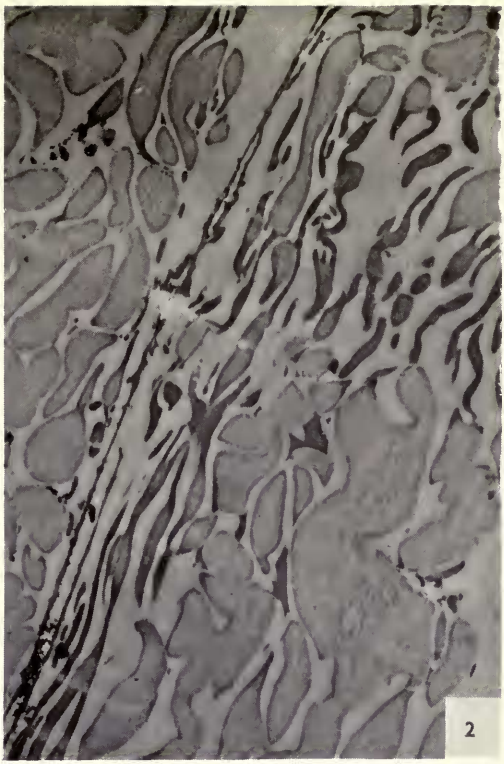
FIGS. 1, 2. Sections of wall of large, presumably fertile megaspore. Fig. 1, × c. 1,500. Fig. 2m × c.1,800.

Dicranum scoparium Hedw. p. 250

FIG. 3. Section of acetolyzed spore. ×10,000.

FIG. 4. Section of part of acetolyzed capsule showing electron-dense droplets concentrated in zone adjacent to remnants of capsule wall and randomly distributed between spores. ×c. 2,000. Section stained with potassium permanganate.


1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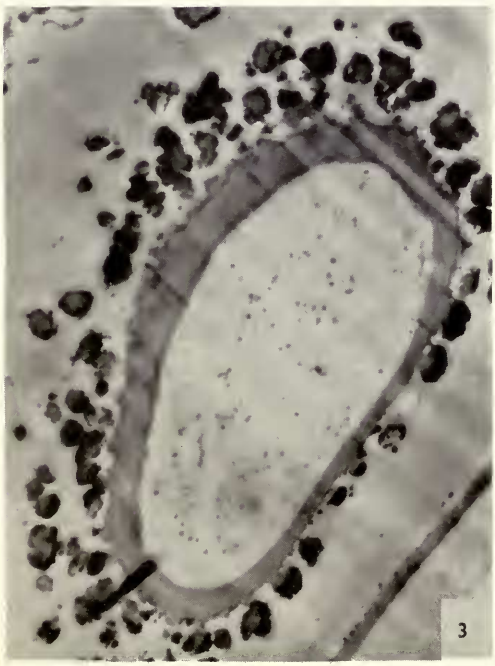
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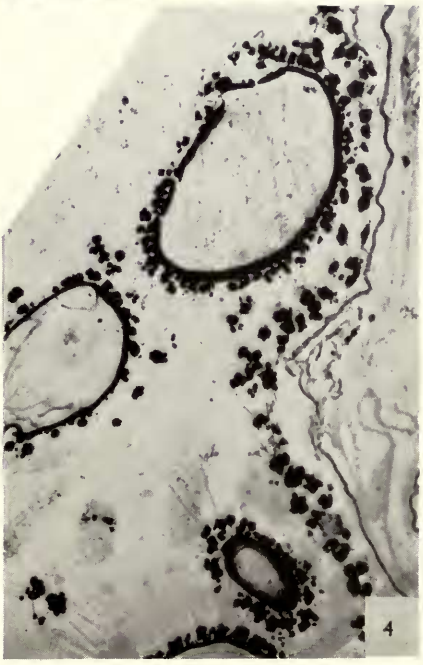

4

PLATE 21

Didymosporites scotti Chaloner p. 249

FIGS. 1–3. Sections of wall of large, presumably fertile megaspores. Fig. 1 is an electron micrograph, Figs. 2, 3 are anoptral contrast pictures. Fig. 1, ×11,250. Figs. 2, 3, ×1,600.

FIG. 4. Complete tetrad from the Lower Carboniferous of Fife, Scotland. Abortive spores can be seen on left of figure between two large, presumably fertile spores. ×120.

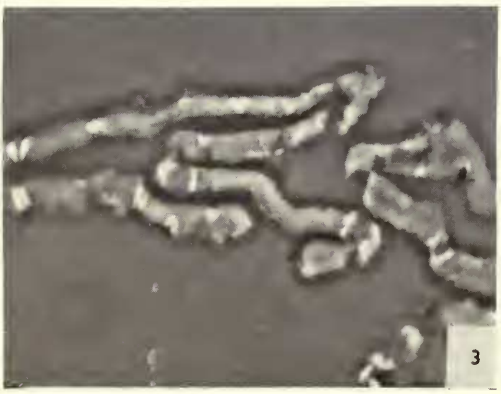
Salvinia auriculata Aubl. p. 251

FIG. 5. Mass of resistant material containing microspores released from an acetolyzed microsporangium. ×254.


1


2


4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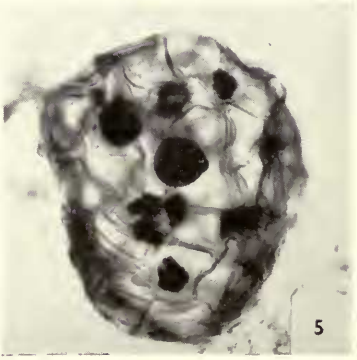
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